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(54) Coronavirus, nucleic acid, protein, and methods for the generation of vaccine, medicaments and diagnostics

(57) A new coronavirus is disclosed herein with a tropism that includes humans. Means and methods are provided for diagnosing subjects (previously) infected with the virus. Also provided are among others vaccines, medicaments, nucleic acids and specific binding members.

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Description

[0001] The invention relates to the fields of virology and medicine. More in particular the invention relates to the identification of a new coronavirus and to means and methods associated with a virus such as means and methods for typing the virus in various samples and diagnosing of disease, means and methods for developing vaccines and medicaments for the treatment of infected subjects or of subjects at risk thereof.

[0002] Coronaviruses, a genus in the family of Coronaviridae, are large enveloped plus strand RNA viruses. The genomic RNA is 27 to 32 kb in size, capped and polyadenylated. Three serologically distinct groups of coronaviruses have been identified. Within each group, viruses are identified by hosts range and genome sequence. Coronaviruses have been identified in mice, rats, chickens, turkeys, swine, dogs, cats, rabbits, horses, cattle and humans (39, 40). Most coronaviruses infect only one host species and can cause severe disease including gastroenteritis, and respiratory tract diseases. In humans, 3 coronaviruses have been studied in detail. HCoV-229E and HCoV-OC43 have been identified in the mid sixties and are known to cause common cold (13-17, 19, 41, 42). Besides common cold it has been suggested that the HCoV-229E may cause a more serious disease in infants as HCoV-229E virus has been isolated from infants suffering from lower respiratory tract disease(28). The third and most recently identified coronavirus: SARS-CoV, is, with its ability to cause a life threatening pneumonia (43), the most pathogenic human coronavirus identified thus far. It has been suggested that SARS-CoV is the first member of a fourth group of coronaviruses, or that the virus is an outlier of the group 2 coronaviruses (27, 44).

The genome of coronaviruses encodes four structural proteins: the spike protein, the membrane protein, the envelope protein and the nucleocapsid protein. Several non-structural proteins are involved in replication and transcription, which are encoded by two long overlapping open reading frames (ORFs) at the 5'end of the genome (1A and 1B). These 2 ORFs are connected via a ribosomal frame shift. The polypeptides encoded by ORF 1A and 1B are post-translationally processed by viral encoded proteases. Furthermore, additional non-structural proteins are encoded between the S and E gene, or between the M and N gene or downstream of the N gene. Some of these "accessory non-structural protein genes" have been found to be not essential for virus reproduction(45, 46). The coronavirus gene products of 1A and 1B are translated from the genomic RNA but the remaining viral proteins are translated from subgenomic mRNAs (sg mRNA), each with a 5'end derived from the 5' part of the genome. The sg mRNA are derived via a discontinuous transcription process that most probably occurs during negative strand synthesis (47). Discontinuous transcription requires base-pairing between cis-acting elements, the transcription associated sequences (TRSs), one located at the 5' part of the genome (the leader TRS) and others located upstream of the ORFs (the body TRSs)(48)).

[0003] The novel coronavirus that we present here was isolated from a child suffering from bronchiolitis. Infection by this virus was not an isolated case since we found 7 more persons suffering from respiratory tract disease carrying the virus. In addition, we show here the complete genome sequence providing critical information concerning the genome structure of the new coronavirus.

[0004] To date there is a range of human diseases with unknown etiology. For many of these a viral origin has been suggested, emphasizing the importance of a continuous search for new viruses[22, 23, 24]. Major difficulties are encountered when searching for new viruses. First, some viruses do not replicate in vitro, at least not in the cells that are commonly used in viral diagnostics. Second, for those viruses that do replicate in vitro and that cause a cytopathic effect (CPE), the subsequent virus-identification methods may fail. Antibodies raised against known viruses may not recognize the cultured virus and virus specific PCR methods may not amplify the new viral genome. We have developed a method for virus discovery based on the cDNA amplified restriction fragment length polymorphism technique (cDNA-AFLP). With this technique, RNA or DNA is reproducibly amplified. There is no need to have prior knowledge of the sequence of the target gene[1]. Generally the cDNA-AFLP method is used to monitor differential gene expression, however, we modified this method such that it can amplify viral sequences either directly from patient blood-plasma/serum samples or indirectly from CPE-positive virus culture (Figure 1). In the modified Virus-Discovery-cDNA-AFLP (VIDISCA) method the mRNA isolation step prior to amplification is replaced by a treatment to selectively enrich for viral nucleic acid. Of relevance to the purification is a centrifugation step to remove residual cells and mitochondria. In addition, a DNAse treatment can be used to remove interfering chromosomal and mitochondrial DNA from degraded cells whereas viral nucleic acid is protected within the viral particle. Finally, by choosing frequently cutting restriction enzymes, the method can be fine-tuned such that most viruses will be amplified.

[0005] In January 2003 a 7-month-old child appeared in the hospital with coryza, conjunctivitis and fever. Chest radiography showed typical features of bronchiolitis and a nasopharyngeal aspirate specimen was collected (sample nr: NL63) five days after the onset of disease. All diagnostic tests on this sample for respiratory syncytial virus (RSV), adenovirus, influenza A and B virus, parainfluenza virus type 1, 2 and 3, rhinovirus, enterovirus, HCoV-229E and HCoV-OC43 were negative. Immunofluorescent assays to detect RSV, adenovirus, influenza A and B virus, and parainfluenza virus type 1, 2 and 3 in cultures of the virus remained negative. Acid lability and chloroform sensitivity tests demonstrated that the virus was most likely enveloped and not a member of the Picornavirus group. In fact it was a new coronavirus.

[0006] In the present invention we present a detailed description of a novel human coronavirus. Coronaviruses are characterized by a very long non-segmented, single-stranded, (+) sense RNA of approximately 27-31 kb. This is the longest genome of any known RNA virus. The genome has a 5' methylated cap and 3' poly-A and functions directly as mRNA. Thus far only 3 human coronaviruses have been characterized, therefore sorting out the characteristics of a fourth human coronavirus supplies attractive information on the variation among the human coronaviruses. The novel virus is a member of the group 1 coronaviruses and is most related to HCoV-229E, yet the differences are prominent. The similarity is not larger than 85% at the nucleotide level, at the position of the 4A and 4B gene of HCoV-229E only one ORF is present in HCoV-NL63 (ORF 3), and the 5' region of the S gene of HCoV-NL63 contains a unique in frame insertion of 537 nucleotides. Since binding of the receptor has been mapped to the N-terminal part of the protein, the 179 amino acids encoded by the insertion are most likely involved in receptor binding. This unique part at the N-terminus of the spike protein might explain the expanded host range of the virus in cell culture. Where HCoV-229E is fastidious in cell culture with a narrow host range, HCoV-NL63 replicates efficiently in monkey kidney cells. Besides HCoV-NL63 also SARS-CoV is able to replicate in monkey kidney cells (Vero-E6 cells and NCI-H292 cells for SARS-CoV (21)). Yet, comparing the predicted Spike genes did not identify a protein region that is shared by both viruses to clarify the common host range of the viruses in *vitro.* Also the insertion in the S gene of HCoV-NL63 was not present in the SARS S gene. Alternatively, other viral proteins may be involved in the cell tropism of a virus, however we did not identify any gene of HCoV-NL63 that had more similarity at the protein level to the SARS-CoV than to the similarity to HCoV-229E.

[0007] The 2 major differences between HCoV-229E and HCoV-NL63: the insertion in the S gene and the altered non-structural accessory proteins genes, are comparably to the differences that are noted between the porcine coronaviruses PRCoV and TGEV. Although these 2 porcine viruses are antigenically and genetically related their pathogenicity is very different. TGEV causes severe diarrhea with a high mortality in neonatal swine. It replicates and destroys the enterocytes in the small intestine whereas PRCoV has a selective tropism for respiratory tissue with very little to no replication in intestinal tissue. The genome differences in the S, 3A and 3B genes between TGEV and PRCoV are comparable with the differences between HCoV-NL63 and HCoV-229E. Alike HCoV-NL63, TGEV has a unique in frame insertion at the 5' part of the S gene ranging from 672 to 681nt (53). Furthermore, the accessory protein genes 3A and 3B that are intact in TGEV, are often mutated or inactive in the PRCoV. Extrapolating these data to the human coronaviruses one can speculate that HCoV-NL63 might be a more pathogenic human virus in comparison with HCoV-229E. However there are no epidemiological data supporting this. Based on our data it seems likely that HCoV-NL63 and HCoV-229E share the same pathogenicity. The common cold virus HCoV-229E can cause a more serious disease in infants (28), comparable to our data that suggest that HCoV-NL63 is causing a respiratory disease only in infants and immuno-compromised patients.

[0008] To date, a viral pathogen cannot be identified in a substantial portion of respiratory disease cases in humans (on average 20%[59]), our data indicate that in a part of these cases HCoV-NL63 is involved. The frequency with which HCoV-NL63 was detected in patients suffering from respiratory disease was up to 5% in January 2003. The virus was not detected in any of the samples collected in the spring or summer of 2003, which is in harmony with the epidemiology of human coronaviruses that have a tendency to spread predominantly in the winter season (15). The primers for our diagnostic PCR were located in the 1B gene and the genomic RNA can be used as template. Using primers that anneal in the nucleocapsid gene or 3'UTR supplies more template in the PCR because besides the genomic RNA also all sg mRNA in infected cells are template for amplification. It might be that the number of persons that we found positive for HCoV-NL63 is an underestimation of the correct number of persons carrying HCoV-NL63.

[0009] The newly found coronavirus, (designated HCoV-NL63) was characterized and sequenced. A sequence of a prototype HCoV-NL63 is provided in figure 19 and parts thereof in table 3. In one aspect the invention therefore provides an isolated and/or recombinant nucleic acid comprising a sequence as depicted in figure 19 and/or table 3, or a functional part, derivative and/or analogue thereof. The virus HCoV-NL63 is characterized by the prototype, however, many natural variants exist as for instance shown in figure 16 for polymorphisms in the ORF 1a region. The existence of such natural variants is normal for RNA viruses that undergo frequent mutation through for instance the introduction of mistakes by the polymerases that copy the genome. HCoV-NL63 viruses that have a slightly divergent nucleic acid sequence are thus also provided by the present invention. Such viruses are considered to be a derivative of the nucleic acid having the prototype nucleic acid sequence. The variant does not necessarily have to be a natural variant. It is very well possible to generate variants through recombinant means. For instance many parts of the virus can be altered through nucleotide substitution to make use of the redundancy in the triplet genetic code for particular amino acids. Thus without altering the amino acid sequence of the encoded proteins. However, even amino acid alterations can typically be introduced without affecting the replicating and coding potential of the viruses. For instance conservative amino acid substitutions are often tolerated. Alterations in the prototype virus may be up to 70% of the nucleic acid sequence without altering the replicating potential of the virus. Thus in one embodiment the invention provides an isolated and/or recombinant nucleic acid that is at least 70% homologous to a nucleic acid of the prototype HCoV-NL63. Most of the viable variants however are at least 95% homologous and more preferably at least 99% to a nucleic acid according to the prototype HCoV-NL63. The homology between different coronaviruses in the UTR regions is typically

high, for this reason the homology in this application is measured in a region outside the UTR regions, preferably in a protein coding region. Thus the invention provides a derivative of HCoV-NL63 virus comprising at least 95% homology and preferably at least 99% homology (on the nucleic acid level) in at least one protein coding region depicted figure 20, 21, 22, 23, or table 3. The nucleic acid of the virus or parts thereof can be cloned and used as a probe to detect the virus in samples. Thus the present invention further provides an isolated and/or recombinant nucleic acid comprising a stretch of 100 consecutive nucleotides of a nucleic acid of the prototype virus, or a region that is at least 95% and preferably at least 99% homologous to said 100 consecutive nucleotides(when measured on the nucleic acid level outside a UTR region). A stretch of 100 consecutive nucleotides is considered to be a functional part of the virus of the present invention. Further provided is a bacterial vector comprising a nucleic acid of HCoV-NL63 or a functional part, derivative and/or analogue thereof. Further provided is a bacterium comprising said bacterial vector. The sequence of HCoV-NL63 or a part thereof can be used to generate a primer that is specific for HCoV-NL63 and thus capable of specifically replicating HCoV-NL63 nucleic acid. Similarly, a probe can be generated that specifically hybridizes to HCoV-NL63 nucleic acid under stringent conditions. Thus the invention further provides a primer and/or probe, capable of specifically hybridizing to a nucleic acid of a HCoV-NL63 virus or functional part, derivative or analogue thereof. Preferably, said primer or probe is capable of hybridizing to said nucleic acid under stringent conditions. In a particularly preferred embodiment said primer and/or probe comprises a sequence as depicted in table 3, table 7, table 10 or figures 16 to 18.

The nucleic acid of the prototype virus encodes various proteins and polyproteins. These proteins are expressed for instance in cells producing the virus or transformed with a nucleic acid encoding the (poly)protein. The invention thus further provides an isolated and/or recombinant proteinaceous molecule comprising a sequence as depicted in figure 20, 21,22, 23 or table 3, or a functional part, derivative and/or analogue thereof. Many different variants of the proteins having the same function in kind, not necessarily in amount are, as mentioned above, present in nature and can be generated artificially, thus the invention further provides an isolated and/or recombinant proteinaceous molecule that is at least 70% homologues to a proteinaceous molecule mentioned above. Such homologous proteins are considered derivatives of a protein encoded by the prototype. Preferably, a derivative protein comprises at least 95% and more preferably at least 99% homology with a protein encoded by the prototype HCoV-NL63. Fragments and parts of a proteinaceous molecule encoded by the prototype virus can be generated, such parts are therefore also provided by the present invention. In a preferred embodiment is provided an isolated and/or recombinant proteinaceous molecule comprising a stretch of at least 30 consecutive amino acids of a proteinaceous molecule encoded by the prototype virus. A protein encoded by the prototype virus can be encoded through a variety of different nucleic acid sequences using the redundancy of the genetic code. Thus the invention further provides a nucleic acid encoding a protein depicted in figure 20, 21,22, 23 or table 3.

The HCoV-NL63 virus can be replicated using in vitro growing cell lines. The virus can be harvested from such cultures and used in a variety of different application including but not limited to the generation of an immune response in a subject. The invention thus further provides an isolated or recombinant virus comprising a HCoV-NL63 nucleic acid sequence or a functional part, derivative and/or analogue thereof. Also provided is an isolated or recombinant virus comprising a proteinaceous molecule as depicted in figure 20, 21 ,22, 23 or table 3, or a functional part, derivative and/or analogue thereof. Subjects that have become infected with HCoV-NL63 can display a number of different clinical and/or subclinical symptoms. Thus further provided is an isolated or recombinant virus or a functional part, derivative or analogue thereof capable of inducing a HCoV-NL63-related disease.

The virus comprises substances that can be used to generate specific binding partners that are able to specifically bind the substance of the virus. Binding partners can be generated by means of injection of the virus into in an immunocompetent subject. As a result of the immunization the serum obtained from the subject will typically contain a number of different antibodies specific for the virus or an immunogenic part, derivative and/or analogue thereof. Specific binding partners can of course be generated through a large variety of different technologies. For instance phage display technologies. The method of producing the specific binding partner is not limited herein. The binding is typically specific for a proteinaceous part of the virus. But can of course also be specific for a virus specific post translation modification of a protein contained in the virus. Thus the present invention further provides an isolated binding molecule capable of specifically binding a proteinaceous molecule of a HCoV-NL63 virus, preferably against encoded by a nucleic acid of the prototype HCoV-NL63. Preferably, a proteinaceous molecule as depicted in figure 20, 21 ,22, 23 or table 3, or a functional part, derivative and/or analogue thereof. The binding molecule can be capable of specifically binding a nucleic acid sequence of a HCoV-NL63, preferably of figure 19 or table 3. The binding molecule is preferably a proteinaceous molecule. However, other binding molecules are also within the scope of the present invention. For instance, it is possible to generate protein mimetics or analogues having the same binding quality as a protein in kind not necessarily in amount. Provided is further a method for producing a binding molecule according to the invention comprising

- producing molecules capable of binding a HCoV-NL63 virus or functional part, derivative or analogue thereof or an isolated and/or recombinant proteinaceous molecule encoded by a prototype nucleic acid of HCoV-NL63, and

- selecting a proteinaceous binding molecule that is specific for said virus and/or said proteinaceous molecule.

[0010] The overall homology of HCoV-NL63 virus with other human coronaviruses is not very high. Thus many different binding molecules capable of specifically binding to HCoV-NL63 virus can be generated. Such binding molecules can be used to detect HCoV-NL63 virus in a sample. The invention thus further provides an isolated or recombinant virus which is immunoreactive with a binding molecule capable of specifically binding HCoV-NL63 virus. Similarly, the invention provides the use of an isolated and/or recombinant proteinaceous molecule as depicted in figure 20, 21,22, 23 or table 3, or a functional part, derivative and/or analogue thereof, for detecting a binding molecule capable of specifically binding HCoV-NL63 virus, or functional part, derivative and/or analogue of said virus in a sample Vise versa, HCoV-NL63 virus can be used to detect a molecule capable of specifically binding said virus in a sample. Binding of HCoV-NL63 virus to a susceptible target cell occurs via a specific receptor. This receptor can be used as a binding molecule of the invention. Preferably, the binding molecule comprises an antibody or functional equivalent thereof. The detection methods can be used to diagnose HCoV-NL63 related disease in a subject. Thus provided is a method for detecting a HCoV-NL63 virus or functional part, derivative or analogue thereof in a sample, comprising hybridizing and/or amplifying a nucleic acid of said virus or functional part, derivative or analogue with a HCoV-NL63 specific primer and/or probe and detecting hybridized and/or amplified product. Further provided is a kit, preferably a diagnostic kit comprising a HCoV-NL63 virus or functional part, derivative or analogue thereof, a binding molecule according to the invention, and/or a HCoV-NL63 virus specific primer/probe according to invention.

[0011] In a particular preferred embodiment is provided the use of a primer or probe capable of specifically hybridizing to a nucleic acid of a HCoV-NL63 virus or functional part, derivative or analogue thereof or a binding molecule capable of specifically binding a proteinaceous molecule depicted in figure 20, 21 ,22, 23 or table 3 or an HCoV-NL63 virus and/or a nucleic acid or functional part, derivative or analogue of a prototype HCoV-NL63 for detecting and/or identifying a HCoV-NL63 coronavirus in a sample. Preferably said nucleic acid comprises a sequence as depicted in table 3.

[0012] The invention further provides a vaccine comprising HCoV-NL63 virus or functional part, derivative or analogue thereof. Further provided is a vaccine comprising a proteinaceous molecule depicted in figure 20, 21 ,22, 23 or table 3 or functional part, derivative and/or analogue of such a proteinaceous molecule. A proteinaceous molecule of the invention may be provided as a vaccine by itself or as a part of the protein or as derivatives or analogues thereof. A suitable analogue is a nucleic acid encoding a HCoV-NL63 virus proteinaceous molecule or a functional part or derivative thereof. The nucleic acid may be used in a DNA vaccine approach which is also provided in the present invention. As carrier for the DNA vaccine it is often suitable to incorporate an expressible HCoV-NL63 virus nucleic acid in a viral replicon allowing replication of the HCoV-NL63 virus nucleic acid in the target cell and thereby allowing boosting of the provided immune response. A HCoV-NL63 virus encoded protein that is suited for such a DNA vaccine approach is the S protein depicted in figure 22 or a functional part, derivative and/or analogue thereof.. A part of an S protein preferably comprises an immunogenic part of the 537 in frame insertion as compared with HCoV-229E virus. Preferably said part comprises essentially said 537 insertion. With the 537 insertion is meant a sequence corresponding to sequences 20472 to 21009 of figure 19. Other suitable candidates are the M and or the N protein or a functional part, derivative and/or analogue thereof. Typically a vaccine includes an appropriate adjuvant. Apart from the use in a vaccine the mentioned virus and/or proteinaceous molecules can also be used to generate and/or boost a HCoV-NL63 virus specific immune response in a subject. The immune response can be both cellular or humoral. Thus further provided is an isolated T-cell comprising a T-cell receptor that is specific for HCoV-NL63 virus or a proteinaceous molecule encoded by a prototype HCoV-NL63 virus. Further provided is an isolated B-cell producing an antibody specific for HCoV-NL63 virus or a proteinaceous molecule encoded by a HCoV-NL63 virus. The antibody or T-cell receptor can be cloned whereupon a cell line can be provided with an expression cassette comprising the cloned receptor or antibody. Thus the invention further provides a cell producing such a receptor or antibody. Such a cell is preferably a cell that is suitable for large scale production of the mentioned proteins such as CHO cells.

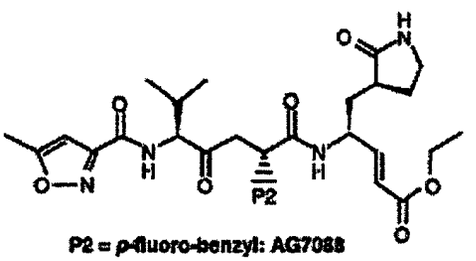
[0013] It is also possible to provide a subject with passive immunity to HCoV-NL63 virus. To this end the subject can be provided with a HCoV-NL63 specific binding molecule of the invention. Such immunity can be used to provide a barrier for (further) infection with HCoV-NL63 virus in the subject, thus further provided is a vaccine comprising a HCoV-NL63 virus specific binding molecule according to the invention. In a preferred embodiment, passive immunity is provided by a human or humanized antibody capable of specifically binding a HCoV-NL63 virus of the invention. The barrier does not have to be perfect. The presence of a binding molecule at least reduces the spread of the virus to other target cells in the subject. The passive immunity may be administered to a subject as prophylactic to at least reduce the spread of HCoV-NL63 virus in the subject when exposed to the virus. Alternatively, the passive immunity may be provided to a subject already infected with the virus. In the latter case one or more HCoV-NL63 virus specific binding molecules of the invention are used as a medicament to at least reduce the spread of the virus in the subject and thereby at least in part combat the virus infection. The invention thus further provides a medicament comprising a HCoV-NL63 virus specific binding molecule according to the invention. Further provided is the use of a virus of the invention or functional part, derivative or analogue thereof or a proteinaceous molecule of the invention or a HCoV-NL63

virus specific binding molecule of the invention, for the preparation of a vaccine against a coronaviral genus related disease.

Further provided is a method for treating an individual suffering from, or at risk of suffering from, an HCoV-NL63 related disease, comprising administering to said individual a vaccine or medicament according to the invention. In yet another embodiment is provided a method for determining whether an individual suffers from an HCoV-NL63 related disease, comprising obtaining a sample from said individual and detecting a HCoV-NL63 virus or functional part, derivative or analogue thereof in said sample.

[0014] In yet another embodiment is provided an isolated cell, or recombinant or cell line comprising HCoV-NL63 virus, or a functional part, derivative and/or analogue thereof. Preferably said cell is a primate cell, preferably a monkey cell. In a preferred embodiment, said cell is a cell that replicates the HCoV-NL63 virus of the invention. In a particular embodiment the cell is a kidney cell. The cell can be used to produce the HCoV-NL63 virus of the invention or to attenuate HCoV-NL63 such that it becomes less pathogenic. Virus attenuation is spontaneous upon continued culture of the virus on the mentioned preferred cell lines. Attenuated HCoV-NL63 virus can be used as a vaccine.

[0015] HCoV-NL63 virus encodes an endoprotease. A sequence for the protease in the prototype HCoV-NL63 virus is depicted in figure (21). The protease is important for the processing of the polyproteins encoded by HCoV-NL63. The action of the protease is at least in part inhibited by a viral protease inhibitor as further described herein. Thus the invention further provides a compound for at least in part inhibiting HCoV-NL63 virus replication. Preferred compounds are inhibitors of inosine monophosphate dehydrogenase (55) (e.g. Ribavirin(54) and mycophenolic acid), orotidine-5'-phosphate decarboxylase inhibitors (e.g. 6-azauridine and pyrazofurin), 3CL-protease inhibitors(56) (e.g. the VN-STLQ-AG7088 ester, see below), cap-methylase inhibitors(58) (carboxylic adenosine analogs e.g. Neoplanocin A and 3-deazaneoplancin A), nitrous oxide synthase inducing compounds (e.g. glycyrrhizin) and Interferons (57). Of these the protease inhibitors are particularly preferred. The sequence VNSTLQ is the N-terminal proteolytic processing site of SARS-3CLpro that is used in the 3Clpro inhibitor VNSTLQ-AG7088 (56). In this compound the hexapeptide VNSTLQ is C-terminally linked to the vinylogous ethyl ester (AG7088, see structural formula 1 depicted below,) that inhibits SARS 3CLpro activity.



Structure of formula I

[0016] The hexapeptide VNSTLQ corresponds to YNSTLQ in HCoV-NL63. Therefore YNSTLQ- AG7088 inhibits the HCoV-NL63 3CLpro orthologs. Thus in a preferred embodiment the protease inhibitor comprises the amino acid sequence VNSTLQ more preferably YNSTLQ. Analogues of such protease inhibitors that comprise the same activity in kind not necessarily in amount are also provided by the present invention. Such analogues include, compounds comprising a peptide with the preferred sequence, wherein the peptide comprises a modification. Other analogues include compounds having protein mimetic activity that mimic the preferred amino-acid sequence.

[0017] S-adenosylmethionine-dependant ribose 2'-orthomethyltransferase Plays a role in the methylation of cap structure (GpppNm) at the 5'end of the viral RNA. Antiviral compounds inhibiting this transfer of methyl groups to reaction (carboxylic adenosine analogs e.g. Neoplanocin A and 3-deazaneoplancin A) interfere with expression of viral proteins.

[0018] The invention further provides a proteinaceous molecule encoded by HCoV-NL63 nucleic acid, wherein said proteinaceous molecule is a 3CL protease or a functional equivalent thereof. Functional equivalents include an proteolytically active part and/or derivative having one or more conservative amino acid substitutions. There are many methods known in the art to determine whether a compound has anticoronaviral activity, preferably antiproteolytic activity of a coronavirus. The invention thus further provides a method for determining whether a compound comprises anticoronavirus replication activity characterized in that said method utilizes HCoV-NL63-virus or a HCoV-NL63 protein involved in replication of HCoV-NL63 or a functional part, derivative and/or analogue thereof. Preferably, the invention provides a method for determining whether a compound is capable of at least in part inhibiting a viral protease char-

acterized in that said protease is a 3CL protease of HCoV-NL63 or a functional part, derivative and/or analogue thereof. Preferred compounds that can be tested for 3CL inhibiting quality are hexapeptides located N-terminally of 3Clpro cleavage sites. Compounds effective in at least in part inhibiting 3Cl proteolytic activity can be used for the preparation of a medicament for the treatment of an individual suffering or at risk of suffering from a HCoV-NL63 virus infection.

[0019] One or more of the preferred anticoronaviral replication compounds can be used as a medicament for the treatment of a subject suffering from or at risk of suffering from a HCoV-NL63 virus infection. The invention thus further provides a medicament for the treatment of an individual suffering from an coronavirus infection or an individual at risk of suffering there from comprising wherein said coronavirus comprises a nucleic acid sequence of a HCoV-NL63 prototype virus or a functional part, derivative and/or analogue thereof.

[0020] In the present invention several different recombinant viruses are produced using HCoV-NL63 virus nucleic acid as a backbone. Such replication competent or replication defective recombinant virus can be used for instance as gene delivery vehicles. On the other hand parts of a HCoV-NL63 virus can be used in gene delivery vehicles that are based on other means for delivering genetic material to a cell. Thus the invention further provides a gene delivery vehicle comprising at least part of a HCoV-NL63 virus nucleic acid. Preferably of the prototype virus. Preferably comprising a nucleic acid encoding a protein of HCoV-NL63 virus or a functional part, derivative and/or analogue thereof. The invention also shows chimearic coronaviruses comprising nucleic acid derived from at least two coronaviruses wherein at least one of said parts is derived from a HCoV-NL63 virus. Said HCoV-NL63 virus derived part comprises preferably at least 50 nucleotides of a protein coding domain. More preferably said HCoV-NL63 derived part comprises at least 500 and more preferably at least 1000 nucleotides of the sequence as depicted in figure 19 or a functional derivative thereof. In a preferred embodiment the invention provides a chimearic coronavirus comprising at least 1000 nucleotides of a sequence as depicted in figure 19 and at least 1000 nucleotides of another coronavirus wherein said latter 1000 nucleotides comprise a sequence that is more than 5% sequence divergent with a sequence as depicted in figure 19. The sequences of a number of HCoV-NL63 virus fragments are depicted in table 3. The location of the fragments in the large genomic RNA is depicted in figure 5. The invention therefore, in one aspect, provides an isolated or recombinant virus comprising a nucleic acid sequence as depicted in table 3, or a functional part, derivative or analogue of said virus. With the aid of the identifying prototype fragments it is possible to further sequence the genome. One way of doing this by primer walking on the genome. A primer is directed to a region of which the sequence is known and this primer is used to sequence a flanking region that is as yet unknown. A subsequent primer can be generated against the newly identified sequence and a further region can be sequenced. This procedure can be repeated until the entire sequence of the virus is elucidated. As a source of the virus one may turn to Dr. C. van der Hoek, Department of Human Retrovirology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands.

[0021] Alignments of the determined nucleic acid sequences revealed the reading frame used in the sequences found, accordingly the invention further provides an isolated or recombinant virus comprising an amino acid sequence as depicted in (table 3). or a functional part, derivative or analogue of said virus. A particular amino acid sequence can be produced from a variety of nucleic acids depending on the codons used. Thus the invention further provides a nucleic acid encoding an amino acid sequence as depicted in (table 3). Further provided is an isolated or recombinant virus comprising a nucleic acid sequence encoding an amino acid sequence as depicted in (table 3), or a functional part, derivative or analogue of said virus.

[0022] Coronaviruses as many other types of viruses acquire a plurality of spontaneous and selected mutations upon spreading of the virus through the subject population and/or during culturing ex vivo. Moreover, artificial mutations having no recognized counterpart in nature can be introduced into the sequence of the prototype virus or a derivative thereof, without altering the viral- and/or disease causing properties of the virus. Having characterized the prototype of the newly discovered subtype gives access to this group of viruses belonging to the same subtype. Thus the invention further provides an isolated or recombinant virus comprising a nucleic acid sequence that is approximately 80% homologous to a sequence as depicted in table 3, or 80 % homologous to an amino acid sequence depicted in Table 3 (. Preferably the homology is at least 90%, more preferably at least 95% and even more preferably at least 99%.

[0023] The respective prototype fragments were compared with a database of viral sequences and hits having a particularly high homology are mentioned in the tables 5 and 6. It may be noted that the compared fragments do not share extensive homology with any of the currently known Coronaviruses. The invention thus provides an isolated and/or recombinant virus comprising an amino acid sequence which is more than 89% homologous to 163- 2 amino acid sequence as depicted in Table 3. Preferably said homology is at least 90%, more preferably at least 95% and even more preferably at least 99%.

Further provided is an isolated or recombinant virus comprising an amino acid sequence which is more than 60 % homologous to 163- 4 amino acid sequence as depicted in Table 3. Preferably said homology is at least 90%, more preferably at least 95% and even more preferably at least 99%.

Further provided is an isolated or recombinant virus comprising a nucleic acid sequence which is more than 85 % homologous to 163- 9 nucleic acid sequence as depicted in Table 3. Preferably said homology is at least 90%, more

preferably at least 95% and even more preferably at least 99%.

Further provided is an isolated or recombinant virus comprising an amino acid sequence which is more than 94 % homologous to 163- 10 amino acid sequence as depicted in Table 3. Preferably said homology is at least 90%, more preferably at least 95% and even more preferably at least 99%.

Further provided is an isolated or recombinant virus comprising an amino acid sequence which is more than 50 % homologous to 163- 11 amino acid sequence as depicted in Table 3. Preferably said homology is at least 90%, more preferably at least 95% and even more preferably at least 99%.

[0024] Further provided is an isolated or recombinant virus comprising an amino acid sequence which is more than 87 % homologous to 163- 14 amino acid sequence as depicted in Table 3. Preferably said homology is at least 90%, more preferably at least 95% and even more preferably at least 99%.

Further provided is an isolated or recombinant virus comprising an amino acid sequence which is more than 83 % homologous to 163- 15 amino acid sequence as depicted in Table 3. Preferably said homology is at least 90%, more preferably at least 95% and even more preferably at least 99%.

Further provided is an isolated or recombinant virus comprising an amino acid sequence which is more than 78 % homologous to 163- 18 amino acid sequence as depicted in Table 3. Preferably said homology is at least 90%, more preferably at least 95% and even more preferably at least 99%.

Further provided is an isolated or recombinant virus comprising a nucleic acid sequence which is at least 50 % homologous to a nucleic acid sequence as depicted in Table 3. Preferably said homology is at least 80%, more preferably at least 90%, more preferably at least 95% and even more preferably at least 99%.

[0025] The invention also provides a functional part, derivative and/or analogue of an isolated and/or recombinant HCoV-NL63 virus. A part of a virus can be a membrane containing part, a nucleocapsid containing part, a proteinaceous fragment and/or a nucleic acid containing part. The functionality of the part varies with the application chosen for the part, for instance, part of the virus may be used for immunization purposes. In this embodiment the functionality comprises similar immunogenic properties in kind as the entire virus not necessarily in amount. Another use of the virus is the infectivity of the virus, for instance, for in vitro (or in vivo) culture, in this embodiment the functionality comprises a similar infectivity in kind not necessarily in amount. Many other functionalities may be defined, as there are many different uses for viruses, non-limiting examples are the generation of chimeric viruses, (i.e. with one or more other (corona) viruses, and the generation of viral vectors for vaccination and/or gene therapeutic purposes. Such viruses and/or vectors also contain a functional part of HCoV-NL63 and are thus also encompassed in the present invention. A functional derivative of a virus of the invention is defined as a virus that has been altered such that the properties of said compound are essentially the same in kind, not necessarily in amount. A derivative can be provided in many ways, for instance through nucleotide substitution (preferably "wobble" based), through (conservative) amino acid substitution, subsequent modification, etcetera.

Analogous compounds of a virus can also be generated using methods in the art. For instance, a chimeric virus can be produced, or an HCoV-NL63 virus having a chimeric protein. For instance, HCoV-NL63 can be rendered more immunogenic by generating a cell surface associated fusion protein comprising at least part of an HCoV-NL63 surface protein and a non-HCoV-NL63 immunogenic part. HCoV-NL63 virus comprising such chimeric protein can be used for inducing an enhanced immune response in a host, for instance for vaccination purposes.

As used herein, the term "a virus of the invention" is meant to also comprise a functional part, derivative and/or analogue of said virus.

[0026] The three groups of coronaviruses are associated with a variety of diseases of humans and domestic animals, including gastroenteritis and upper and lower respiratory tract disease. The human coronaviruses HCoV-229E and HCoV-OC43 are associated with mild disease (the common cold) but more severe disease is observed in children [16], albeit at a very low incidence. Several coronaviruses cause a severe disease in animals and SARS-CoV is the first example of a coronavirus that causes severe disease in humans. However, it should be emphasized that a substantial part of respiratory disease cases in humans remains undiagnosed. For instance, a recent survey of respiratory viruses in hospitalized children with bronchiolitis in Canada could not reveal a viral pathogen in about 20% of the patients[17]. The fact that we identified the new coronavirus in a child with bronchiolitis shows that HCoV-NL63 is a pathogenic respiratory virus.

When considering that the HCoV-NL63 is a pathogenic respiratory virus able to cause bronchiolitis in infected children, the interesting question remains why HCoV-NL63 was not recognized previously by cell culture. We found that the virus can be cultured in monkey kidney cells (tMK or LLC-MK2 cells), cells that are often used in a routine diagnostic setting and one might therefore speculate that HCoV-NL63, like SARS-CoV, was newly introduced from an animal reservoir into the human population or that this is a human virus that recently broadened its host cell range. Clearly it is of importance to study the prevalence of HCoV-NL63 infection, and screening specimens from patients with respiratory tract disease using the HCoV-NL63 diagnostic RT-PCR will shed light on this issue.

It is remarkable that the new human coronavirus was harvested from tMK cells and LLC-MK2 cells since coronaviruses are typically fastidious in cell culture with a narrow host range. However, both SARS-CoV and HCoV-NL63 seem to

replicate efficiently in monkey kidney cells (Vero-E6 cells and NCI-H292 cells for SARS-CoV). The recently described genome of SARS-CoV has several exclusive features, including some unique open reading frames that are probably of biological significance[15, 18]. We will therefore analyze the complete genome sequence of HCoV-NL63 to screen for similarities and differences with SARS-CoV that may determine the expanded host cell range and enhanced pathogenicity of these viruses.

[0027] HCoV-NL63 is associated with a particular phenotype in infected subjects. The phenotype can encompass bronchiolitis, coryza, conjunctivitis and fever and may further encompass other respiratory problems and diarrhea. In one embodiment the invention thus further provides an isolated and or recombinant virus of the invention (having one or more of the above mentioned homology) wherein said virus or functional part, derivative and/or analogue further comprises the capability to induce an HCoV-NL63 related disease or symptom in a subject. In another embodiment the invention provides an isolated and/or recombinant virus of the invention further comprising the property to cause CPE in tertiary monkey kidney cells (tMK; Cynomolgus monkey[37]) and/or upon passage onto the monkey cell line LLC-MK2 (ECCAC 85062804, ATCC CCL-7). In a preferred embodiment said virus does not produce CPE in Vero-cells (ATCC CRL-1586)[34].

[0028] The invention further provides a nucleic acid as depicted in table 3, and an amino acid sequence as depicted in Table 3, or a functional part and/or equivalent of such a nucleic acid and/or amino acid sequence. A functional equivalent of said nucleic acid comprises the same hybridization properties in kind, not necessarily in amount, as said nucleic acid (or part thereof). A functional equivalent of an amino acid sequence of the invention comprises the same immunogenic properties in kind, not necessarily in amount, as said amino acid sequence (or part thereof). A part of a nucleic acid of the invention comprises at least 15 nucleotides, preferably at least 20, more preferably at least 30 nucleotides. A part of an amino acid sequence comprises at least 5 amino acids in peptidic linkage with each other, more preferably at least 8, and more preferably at least 12, more preferably at least 16 amino acids. In a preferred embodiment said nucleotides and/or amino acids are at least semi-consecutive, more preferably, said nucleotides and/or amino acids are consecutive. An equivalent of a nucleic acid and/or amino acid sequence of the invention or part thereof comprises at least 80% homology to a nucleic acid and/or amino acid sequence of the invention, preferably at least 90% homology, more preferably at least 95% and even more preferably at least 99% homology to a nucleic acid and/or amino acid sequence of the invention or a part thereof.

[0029] The invention further provides a primer and/or probe, capable of specifically hybridizing to a nucleic acid of a virus or functional part, derivative or analogue according to the invention, preferably a primer and/or probe, capable of specifically hybridizing to a nucleic acid sequence as depicted in Table 3. More preferably, a primer and/or probe, which is capable of hybridizing to said nucleic acid under stringent conditions. In a particular preferred embodiment is provided a primer and/or probe, comprising a sequence as depicted in Table 7.

[0030] The art knows many ways in which a specific binding member can be generated against an identified nucleic acid, lipid and/or amino acid sequence. Such specific binding members may be of any nature but are typically of a nucleic acid and/or proteinaceous nature. The invention thus further provides an isolated molecule capable of specifically binding a virus, nucleic acid and/or amino acid or functional part, derivative or analogue thereof according to the invention. Said isolated molecule is also referred to as specific binding member. Preferably said specific binding member is capable of specifically binding at least part of a nucleic acid sequence as depicted in table 3 and/or at least part of an amino acid sequence as depicted in Table 3. In a preferred embodiment said binding member is a proteinaceous molecule. Preferably an antibody or a functional part, derivative and/or analogue thereof. A specific binding member preferably comprises a significantly better binding property for the HCoV-NL63 virus compared to unrelated control. However, for instance for antibodies, it is possible that the epitope specifically recognized in HCoV-NL63 is also present in a limited number of other molecules. Thus though the binding of the binding member may be specific, it may recognize also other molecules than those present in HCoV-NL63. This cross-reactivity is to be separated from a-specific binding and is a general property of antibodies. Cross-reactivity does not usually hinder the selection of suitable specific binding members for particular purposes. For instance a specific binding member that also recognized a protein in liver cells can be used in many applications even in the presence of liver cells, where additional information such as location in the cell can often be used to discriminate.

[0031] One source of an antibody of the invention is the blood of the infected subjects screened for the virus of the present invention. One may further characterize B-cells obtained from said subject. A suitable B-cell may be cultured and the antibody collected. Alternatively, the antibody may be sequenced from this B-cell and generated artificially. Another source of an antibody of the invention can be generated by immunisation of test animals or using artificial libraries to screen a purified fraction of virus. A functional part of an antibody has essentially the same properties of said antibody in kind, not necessarily in amount. Said functional part is preferably capable of specifically binding an antigen of HCoV-NL63. However, said functional part may bind such antigen to a different extend as compared to said whole antibody. A functional part or derivative of an antibody for instance comprises a FAB fragment or a single chain antibody. An analogue of an antibody for instance comprises a chimeric antibody. As used herein, the term "antibody" is also meant to comprise a functional part, derivative and/or analogue of said antibody.

[0032] Once antibody of the invention is obtained, a desired property, such as its binding capacity, can be improved. This can for instance be done by an Ala-scan and/or replacement net mapping method. With these methods, many different proteinaceous molecules are generated, based on an original amino acid sequence but each molecule containing a substitution of at least one amino acid residue. Said amino acid residue may either be replaced by Alanine (Ala-scan) or by any other amino acid residue (replacement net mapping). Each variant is subsequently screened for said desired property. Generated data are used to design an improved proteinaceous molecule.

[0033] There are many different ways in which a specific binding member can be generated. In a preferred embodiment the invention provides a method for producing a specific proteinaceous binding member comprising producing proteinaceous molecules capable of binding a virus according to the invention or to a functional part, derivative or analogue, and selecting a proteinaceous molecule that is specific for said virus. If need be, the method may be used to generate a collection of proteinaceous molecules capable of binding to said virus or functional part, derivative and/or analogue thereof and selecting from said collection one or more binding members capable of specifically binding said virus or functional part, derivative and/or analogue thereof.

[0034] Any specific binding member is characteristic for the HCoV-NL63virus of the invention. Thus a virus that is specifically reactive with such binding member is an HCoV-NL63 virus and thus provided by the invention. Thus the invention provides an isolated and/or recombinant virus that is immunoreactive with specific binding member of the invention, preferably a proteinaceous binding member. The invention further provides a composition of matter comprising isolated HCoV-NL63 virus, and/or a virus essentially corresponding to HCoV-NL63. The term, a virus "essentially corresponding to HCoV-NL63" refers to HCoV-NL63 viruses which are either identical to the HCoV-NL63 strain described hereinabove, or which comprises one or more mutations compared to the said HCoV-NL63strain. These mutations may include natural mutations or artificial mutations. Said mutations of course should allow detection with a specific binding member of HCoV-NL63, not necessarily with all of the specific binding members). Said mutations should allow the detection of the variants using common detection methods such as antibody interaction, amplification and/or hybridization.

[0035] Considering that specific binding members are important molecules for instance for diagnostic purposes, the invention further provides the use of a virus of the invention or functional part, derivative and/or analogue thereof, for detecting a molecule capable of specifically binding said virus in a sample. Further provided is the use of a nucleic acid and/or amino acid sequence of a virus or functional part, derivative or analogue as defined by the invention, for detecting a molecule capable of specifically binding said virus or functional part, derivative and/or analogue in a sample. Preferably said nucleic acid and/or amino acid sequence comprises a sequence as depicted in table 3 or Table 3 or a functional part, derivative or analogue thereof. Preferably said part is at least 30 nucleotides and/or amino acids long wherein said part preferably comprises more than 95% sequence identity, preferably more than 99%. In a preferred aspect said specific binding member comprises a specific ligand and/or antibody of said virus.

[0036] Further provided is a primer and/or probe according to the invention, a specific binding member of the invention, and/or a nucleic acid of a virus or functional part, derivative or analogue according to the invention, for detecting and/or identifying a HCoV-NL63 coronavirus or part thereof in a sample. Preferably, said nucleic acid comprises a sequence as depicted in table 3.

[0037] HCoV-NL63 virus may be used to generate an immune response in a subject. This can be useful for instance in vaccination strategies. Thus the invention further HCoV-NL63 provides HCoV-NL63 virus or functional part, derivative or analogue thereof for use as a vaccine or medicament. The medicament use is typically when the subject is already infected with the virus and the immunogen is used to augment the immune response against the virus. The invention further provides a specific binding member of the invention for use as a vaccine or medicament. This use is particularly favorable for when the specific binding member comprises a proteinaceous molecule, preferably an antibody or functional part, derivative and/or analogue thereof. Such an antibody can provide passive immunity but may also have active components such as proteases attached to it. The medicament use may again be the case wherein a subject infected with an HCoV-NL63 virus is treated with the specific binding member.

[0038] Vaccines may be generated in a variety of ways. One way is to culture the HCoV-NL63 virus for example on the mentioned monkey cell line(s) and to use inactivated virus harvested from the culture. Alternatively, attenuated virus may be used either inactivated or as a live vaccine. Methods for the generation of coronavirus vaccines may be adapted to produce vaccines for the HCoV-NL63 of the invention. The invention thus further provides the use of an HCoV-NL63 virus or functional part, derivative or analogue thereof for the preparation of a vaccine against a coronaviral genus related disease. The invention further provides the use of a specific binding member of the invention for the preparation of a vaccine or medicament against a coronaviral genus related disease. Further provided is the use of an HCoV-NL63 virus or functional part, derivative or analogue thereof, a specific binding member of the invention, a nucleic acid of the invention or a primer and/or probe of the invention for diagnosis of a coronaviral genus related disease. Preferably said coronaviral genus related disease comprises a HCoV-NL63coronavirus related disease.

[0039] Further provided is a vaccine comprising an HCoV-NL63 virus or functional part, derivative or analogue thereof and/or a specific binding member of the invention. Also provided is a medicament comprising an HCoV-NL63virus or

functional part, derivative or analogue thereof and/or a specific binding member of the invention. Preferably said vaccine or medicament is used for at least in part preventing and/or treating a HCoV-NL63 related disease.

[0040] An important use of the present invention is the generation of a diagnostic tool for determining whether a subject is suffering from an HCoV-NL63 virus infection or has been exposed to an HCoV-NL63 virus infection. Many different diagnostic applications can be envisioned. They typically contain an identifying component allowing the typing of the virus that is or was present in the subject. One diagnostic tool for HCoV-NL63 makes use of the particular proliferation characteristics of the virus in various cell lines. It replicates in the mentioned preferred monkey cell lines but does not replicate in Vero- cells. This property can be used to discriminate HCoV-NL63 from other known coronaviruses. Thus in one aspect the invention provides a diagnostic kit comprising at least one of the preferred monkey cell lines, preferably the tertiary monkey kidney cells (tMK; Cynomolgus monkey or the monkey cell line LLC-MK2. Many modern diagnostic kits comprise a specific binding member (to detect the virus or virus infected cells) and/or an HCoV-NL63 virus or a functional part, derivative and/or analogue thereof and/or amino acid of the invention or a functional part, derivative and/or analogue thereof (for detecting antibodies in blood components of the diagnosed subject). Many other current diagnostic kits rely on identification of HCoV-NL63 virus specific nucleic acid in a sample. There are various ways in which such an assay may be implemented one is a method for detecting an HCoV-NL63 virus or functional part, derivative or analogue thereof in a sample, comprising hybridizing and/or amplifying a nucleic acid of said virus or functional part, derivative or analogue with a primer and/or probe according to the invention and detecting hybridized and/or amplified product. The invention thus also provides a diagnostic kit comprising an HCoV-NL63 virus or functional part, derivative or analogue thereof, a specific binding member according to the invention and/or a primer/probe according to the invention.

[0041] Further provided is a method for treating an individual suffering from, or at risk of suffering from, a HCoV-NL63 related disease, comprising administering to said individual a vaccine or medicament according to the invention. Also provided is a method for determining whether an individual suffers from a HCoV-NL63 related disease, comprising obtaining a sample from said individual and detecting a HCoV-NL63 virus or functional part, derivative or analogue thereof in said sample with a method and/or diagnostic kit of the invention.

[0042] Further provided is an isolated or recombinant nucleic acid encoding a virus or functional part, derivative and/or analogue according to the invention and a nucleic acid according to the invention, comprising at least a functional part of a sequence as depicted in Table 3. Further provided is an amino acid sequence encoded by a nucleic acid according to the invention, and an amino acid sequence according to the invention, comprising at least a functional part of a sequence as depicted in Table 3. Further provided is a proteinaceous molecule capable of specifically binding HCoV-NL63, obtainable by a method according to the invention and, the use of such a proteinaceous molecule in a vaccine or a diagnostic method for the detection of HCoV-NL63.

EXAMPLES

Example 1

cDNA-AFLP for virus discovery

[0043] We modified the cDNA-AFLP technique such that it can amplify viral sequences from blood-plasma/serum samples or from CPE-positive culture supernatants (Figure 1). In the adjusted method the mRNA isolation step prior to amplification is replaced by a treatment to purify viral nucleic acid. Of importance to the purification is a centrifugation step to remove residual cells and mitochondria. In addition, a single DNAse treatment is sufficient to get rid of interfering chromosomal DNA and mitochondrial DNA from broken down cells and finally, by choosing frequent cutting restriction enzymes, the method is fine-tuned such that the majority of viruses will be amplified. With this so-called Virus Discovery cDNA-AFLP (VIDISCA) we were able to amplify viral nucleic acids from EDTA-plasma of a person with hepatitis B virus infection and a person with an acute Parvo B19 infection (results not shown). The technique can also detect HIV-1 in a positive culture supernatant demonstrating its capacity to identify both RNA and DNA viruses (results not shown).

[0044] To eliminate residual cells, 110 µl of virus culture supernatant was spun down for 10 min at maximum speed in an Eppendorf microcentrifuge (13500 rpm). One hundred µl was transferred to a fresh tube and DNAse treated for 45 minutes at 37°C using 15 µl of DNAse buffer and 20 Units of DNAse I (Ambion). The DNAse treatment was included to get rid of chromosomal DNA from broken down cells. After this 900 µl of L6 lysis buffer and 40 µl of silica suspension was added and nucleic acids were extracted as described by Boom[4]. The viral nucleic acids were eluted in 40 µl H_2O. With 20 µl eluate the reverse transcription was performed using 2.5 µg random hexamers (Amersham Bioscience), 200 U MMLV-RT (InVitrogen) in a buffer containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 0.1% Triton X-100, 4.8 mM MgCl2, and 0.4 mM of each dNTP. The sample was incubated at 37°C for 90 minutes. Subsequently the second strand DNA synthesis was performed using 26 U Sequenase II (Amersham Bioscience), 7.5 U RNAse H (Amersham Bioscience) in 0.25 mM dNTPs each, 17.5 mM MgCl2 and 35 mM Tris-HCl pH 7.5. After the incubation at 37°C for 90

minutes a phenol/chloroform extraction was performed followed by an ethanol precipitation. The pellet was dissolved in 30 µl of H_2O. The cDNA-AFLP was performed essentially as described by Bachem[1] with some modifications. The dsDNA was digested with the HinP I and MseI restriction enzymes (New England Biolabs) according to the manufacturers protocol. After the digestion, MseI adaptor and HinP I adaptor (see below) are added together with 5U ligase enzyme (InVitrogen) and ligase buffer, followed by an additional incubation of 2 hrs at 37°C. The MseI adaptor and HinP I adaptor were prepared previously by mixing a top strand oligo for the MSE and the HinP1 adaptors (Table 1) with a bottom strand oligo for the MSE adaptor and for the HinP1 adaptor, incubate at 65° C. followed by cooling down to room temperature in the presence of a 1:40 dilution of ligase buffer.

[0045] The first PCR was performed with 10 µl of ligation mixture as input, 2.5 U of AmpliTaq polymerase (Perkin-Elmer), 100 ng of HinPI standard primer and 100 ng of MseI standard primer. The PCR reaction was performed according to the profile 5min 95C; 20 cycles of: 1min 95°C-1min 55°C-2min 72°C; 10 min 72 °C. Five µl of first PCR product was used as input in the second "selective" amplification step containing 100 ng of HinPI-N primer and 100 ng MseI-N (sequence of the standard primers extended with one nucleotide) and 2 U AmpliTaq polymerase. The selective PCRs were amplified according to the profile of the "touch down PCR": 10 cycles of 60 sec 94° C-30 sec 65° C-1 min 72°C over which the annealing temperature was reduced from 65°C with 1 °C with each cycle, followed by 23 cycles: 30 sec 94°C-30 sec 56 °C-1 min 72 °C. Finally the sample was incubated for 10 min at 72°C. The PCR products were evaluated on 4% Metaphor® gels (Cambrex, Rockland, USA). If the bands on the gel were very faint the PCR products were concentrated by vacuum drying using 60 µl of the PCR product. The PCR fragments of interest were cut out of gel and DNA was eluted from the gel using the Qiagen gel purification kit according to the manufacturer's protocol. The PCR products were cloned using pCR® 2.1-TOPO plasmid (InVitrogen) and chemically competent One Shot E. coli (InVitrogen). A PCR on the colony was performed and this PCR product was input for sequencing the insert using Big Dye terminator chemistry (Applied Biosystems). The reverse transcription step was excluded, only HinP I digestion and adaptor ligation was performed, the first PCR was performed with 35 cycles instead of 20 and those first PCR fragments were visualized on agarose gel electrophoresis.

DNA sequencing and analysis.

[0046] Coronavirus-PCR product containing plasmids were sequenced with the BigDyeTM Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif.), using the -21 M13RP and T7 primers. Electrophoresis of sequencing reaction mixtures was performed with an Applied Biosystems 377 automated sequencer, following the manufacturer's protocols. The Sequence Navigator (version 1.01) and Auto Assembler (version 2.1) software packages (ABI, California, USA) were used to analyze all sequencing data. Sequences were compared to all sequences in the Genbank database using the BLAST tool of the NCBI web page: http://www.ncbi.nlm.nih.gov/blast. For phylogenetic analysis the sequences were aligned using the ClustalX software package[34] with the following settings: Gap opening penalties: 10.00; Gap extension penalty 0.20, Delay divergent sequences switch at 30% and transition weight 0.59. Phylogenetic analysis was carried out using the neighbor-joining method of the MEGA program (9). The nucleotide distance matrix was generated either by Kimura's 2-parameter estimation or by the p-distance estimation (5). Bootstrap resampling (500 replications) was employed to place approximate confidence limits on individual branches.

Determining the nucleotide sequence of the complete HCoV-NL63 genome.

[0047] Using a combination of specific primers, located in the already sequenced domains of the HCoV-NL63 genome, and the proprietary PALM-method (WO 0151661) we are in the process of cloning and determining the full-length genomic sequence for this new coronavirus. Using a combination of 5'-oligonucleotides located in the analyzed part of the HCoV-NL63 genome and a 3' tagged random primer (JZH2R) additional fragments were amplified using a nested RT-PCR protocol similar to the one mentioned previously.

Isolation of SZ 163

[0048] In January 2003 a 7-month-old child appeared in hospital with coryza, conjunctivitis and fever. Chest radiography showed typical features of bronchiolitis and four days after the onset of disease a nasopharyngeal aspirate specimen was collected (sample nr: HCoV-NL63). All routinely used tests on this sample for adenovirus, respiratory syncytial virus (RSV), influenza A and B, parainfluenza 1, 2 and 3, rhinovirus, HCoV-229E and HCoV-OC43 were negative. The clinical sample was subsequently inoculated onto a variety of cells including human fibroblast lung (HFL) cells, tertiary monkey kidney cells (tMK; Cynomolgus) and R-HeLa cells. A CPE was detected exclusively on tMK cells and first noted at eight days post-inoculation. The CPE was diffuse with a refractive appearance in the affected cells followed by cell detachment after 7 days. More pronounced CPE was observed upon passage onto LLC-MK2 cells. Besides overall cell rounding, moderate cell enlargement was observed. Additional subculturing on human endothelial

lung cells, HFL, Rhabdomyosarcoma cells and Vero cells remained negative for CPE. Immunofluorescent assays to detect influenzavirus A and B, RSV, adenoviruses or parainfluenza virus types 1, 2 or 3 in the culture remained negative The culture supernatant of infected LLC-MK2 cells was subsequently analyzed by VIDISCA. As control we used the supernatant of uninfected LLC-MK2 cells. After the second PCR amplification step, several DNA fragments were present in the test sample but not in the control. These fragments were cloned and sequenced. A Blast search in GenBank revealed that 8 of 16 fragments had sequence similarity to the family of corona viruses with the highest homology the human corona virus 229E (Tables 4 and 5).

[0049] Phylogenetic analysis of a 270 nt fragment of the replicase 1B region indicated that we identified a distinct new member of the coronavirus group 1. With the VIDISCA technique, 8 HCOV-163-specific fragments, named 163-2, 163-4, 163-9, 163-10, 163-11, 163-14, 163-15 and 163-18 were isolated, cloned, sequenced and aligned with the relevant sequences from GenBank. The Genbank accession number of the used sequences are: MHV (mouse hepatitis virus): AF201929; HCoV-229E: AF304460; PEDV (porcine epidemic diarrhea virus): AF353511; TGEV (transmissible gastroenteritis virus): AJ271965; SARS-CoV: AY278554; IBV (avian infectious bronchitis virus): NC_001451; BCoV (bovine coronavirus): NC_003045; FCoV (feline coronavirus): Y13921 and X80799; CCoV (canine coronavirus): AB105373 and A22732; PRCoV (porcine respiratory coronavirus): M94097; FIPV (feline infectious peritonitis virus): D32044. Position of the HCoV-NL63 fragments compared to HCoV-229E (AF304460): Replicase 1AB gene: 15155-15361, 16049-16182, 16190-16315, 18444-18550, Spike gene: 22124-22266; Nucleocapsid gene: 25667-25882 and 25887-25957; 3'UTR: 27052-27123. Branch lengths indicate the number of substitutions per sequence. From the most closely related species sequence identity scores were calculated (Tables 5 and 6).

[0050] Also the deduced amino acid sequence were aligned to the corresponding domains in the open reading frames of related corona (-like) viruses (Table 6).

[0051] The human corona viruses account for 10 to 30% of the common colds in man[7], and it is not unusual to find a coronavirus in a child with a respiratory illness. However, it is striking that the virus HCoV-NL63 was harvested from LLC-MK cells. Human Corona virus 229E and OC-43 are known for there inability to replicate on monkey kidney cells. Intriguingly, the newly identified human corona virus that is responsible for SARS is also able to replicate in monkey kidney cells[30].

Propagation of HCoV-NL63 in cell culture

[0052] A nasopharyngeal aspirate was collected 4 days after the onset of symptoms. The specimen was tested for the presence of adenovirus, RSV, influenza A, influenza B, and parainfluenza type 1, 2 an 3 using the Virus Respiratory Kit (Bartels: Trinity Biotech plc, Wicklow Ireland). In addition, PCR diagnosis for rhinoviruses, meta-pneumovirus and HCoV-OC43 and HCoV-229E were performed[2, 10]. The original nasopharyngeal aspirate was subsequently inoculated onto a variety of cell cultures including HFL cells, tMK cells and R-HeLa cells. The tubes were kept in a roller drum at 34°C and observed every 3 to 4 days. Maintenance medium was replenished every 3 to 4 days. Two different types of medium were implemented: Optimem 1 (Gibco) without bovine fetal serum was used for the tMK cells and MEM Hanks' /Earle's medium (Gibco) with 3% bovine fetal serum was used for the remaining cell types. On the virus culture direct staining was performed with pools of fluorescent-labeled mouse antibodies against influenzavirus A and B, RSV and adenoviruses (Imagen, DAKO). Indirect staining was performed for parainfluenza virus types 1, 2 or 3 with mouse antibodies (Chemicon, Brunschwig, Amsterdam Netherlands) and subsequent staining with labeled rabbit anti-mouse antibodies (Imagen, DAKO).

Method to detect HCoV-NL63 in nasopharyngeal swabs.

[0053] For the diagnostic RT-PCR, nucleic acids were extracted by the Boom method[4] 4 from 50 µl virus supernatant or 50 µl suspended nasopharyngeal swab. The reverse transcription was performed as described above with the exception that 10 ng of reverse transcription primer repSZ-RT (Table 7) was used. The entire RT mixture was added to the first PCR mixture containing 100 ng of primer repSZ-1 and 100 ng of primer repSZ-3. The PCR reaction was performed according to the profile 5 min 95 °C; 20 cycles of: 1 min 95°C - 1min 55°C - 2 min 72°C; 10 min 72 °C. A nested PCR was started using 5 µl of the first PCR with 100 ng of primer repSZ-2 and 100 ng of primer repSZ-4. Twenty-five PCR cycles were performed of the same profile as the first PCR.

[0054] Ten µl of the first and 10 µl of the nested PCR was analyzed by agarose gel electrophoresis (Fig. 2). Cloning and sequencing of the fragments was performed essentially as described above.

Method of raising polyclonal antibodies

[0055] Appropriate domains within the HCoV-NL63 surface proteins (e.g. S-glycoprotein or HE- glycoprotein) can be selected and amplified with suitable oligonucleotides and RT-PCR. The corresponding purified viral antigens can

be obtained by expression in a suitable host *(e.g. Yarrowia lipolytica* as previously described[38]). Female NZW rabbits (approx 4 kg) are primed with 0.5 to 5.0 mg of viral protein antigen preparation. The antigen is suspended in 0.5 ml. of phosphate buffered saline (pH 7.3) and emulsified in an equal volume of complete Freund's adjuvant (CFA). Freund's Adjuvant is a well-established adjuvant system that is appropriate for use in these experiments where small amounts of antigen are used, and where immunogenicity of the antigen (although likely) is unknown. Published guidelines for use will be followed, including limiting injection to 0.1 ml at each site, using CFA only for initial immunization dose. This antigen preparation (1 ml total volume) is injected subdermally in the loose skin on the backside of the rabbit's neck. This injection route is immunologically effective and minimizes the possibility of local inflammation associated with unilateral or bilateral flank injection (such ensuing flank inflammation can impair animal mobility). After resting for 3 weeks, one ml of blood will be removed from the ear artery for a test bleed. Antibodies will be boosted if titers of the desirable antibodies are judged to be too low. Rabbits with adequate antibody levels will be boosted subdermally 1.0 mg of antigen contained in CFA. Boosted animals will be bled after two weeks; i.e., 15 ml of blood will be taken from the ear artery using a heat lamp to dilate the blood vessel. The rabbit will be placed in a commercial restraint, tranquillized with xylazine not more than seven times in total after which the rabbit will be exsanguinated by cardiac puncture following anesthesia using xylazine/ketamine.

Method for Vaccine production

[0056] For the production of a subunit vaccine the S-glycoprotein perhaps combined with the HE, M and N proteins, could be expressed in a suitable eukaryotic host (e.g. *Y. lipolytica* or LLC-MK2 cells) and purified using preferentially two small affinity tags (e.g. His-tag or the StrepII tag). After appropriate purification, the resulting viral proteins can be used as a subunit vaccine.

[0057] Alternatively the HCoV-NL63 virus can be propagated in a suitable cell line as described above and subsequently treated as described by Wu [11]. Briefly the virus is precipitated from culture medium with 20% polyethylene glycol 6000 and purified by ultracentrifugation at 80.000 $\times$ g for 4 hours through a discontinuous 40-65% sucrose gradient followed by a linear 5 to 40 % CsCl gradient for 4 hours at 120.000 $\times$ g. The resulting virus preparation can be inactivated by heating for 30 minutes at 65° C as described by Blondel[3].

Analysis of S glycoprotein or any of the HCOV-NL63 viral proteins binding to an immobilized ligand (e.g. antibody) in an optical biosensor.

[0058] Binding reactions were carried out in an IAsys two-channel resonant mirror biosensor at 20°C (Affinity Sensors, Saxon Hill, Cambridge, United Kingdom) with minor modifications. Planar biotin surfaces, with which a signal of 600 arc s corresponds to 1 ng of bound protein/mm2, were derivatized with streptavidin according to the manufacturer's instructions. Controls showed that the viral proteins did not bind to streptavidin-derivatized biotin surfaces (result not shown). Biotinylated antibody was immobilized on planar streptavidin-derivatized surfaces, which were then washed with PBS. The distribution of the immobilized ligand and of the bound S-glycoprotein on the surface of the biosensor cuvette was inspected by the resonance scan, which showed that at all times these molecules were distributed uniformly on the sensor surface and therefore were not micro-aggregated. Binding assays were conducted in a final volume of 30 µl of PBS at 20 $\pm$ 0.1°C. The ligate was added at a known concentration in 1 µl to 5 µl of PBS to the cuvette to give a final concentration of S-glycoprotein ranging from 14 to 70 nM. To remove residual bound ligate after the dissociation phase, and thus regenerate the immobilized ligand, the cuvette was washed three times with 50 µl of 2 M NaCl-10 mM Na2HPO4, pH 7.2, and three times with 50 µl of 20 mM HCl. Data were pooled from experiments carried out with different amounts of immobilized antibody (0.2, 0.6, and 1.2 ng/mm2). For the calculation of k_{on}, low concentrations of ligate (S-glycoprotein) were used, whereas for the measurement of k_{off}, higher concentrations of ligate were employed (1 µM) to avoid any rebinding artefacts. The binding parameters k_{on} and k_{off} were calculated from the association and dissociation phases of the binding reactions, respectively, using the non-linear curve-fitting FastFit software (Affinity Sensors) provided with the instrument. The dissociation constant (K_d) was calculated from the association and dissociation rate constants and from the extent of binding observed near equilibrium.

Example 2

Methods

Virus isolation

[0059] The child, who was living in Amsterdam, was admitted to the hospital with complaints of coryza and conjunctivitis since 3 days. At admission she had shortness of breath and refused to drink. The patient's temperature was 39

°C, the respiratory rate was 50 breaths/min with oxygen saturation of 96% and her pulse was 177 beats/min. Upon auscultation bilateral prolonged expirium and end-expiratory wheezing was found. A chest radiograph showed the typical features of bronchiolitis. The child was treated with salbutamol and ipratropium at the first day, followed by the use of salbutamol only for 5 days. The child was seen daily at the out patient clinic and the symptoms gradually decreased. A nasopharyngeal aspirate was collected 5 days after the onset of symptoms. The specimen was tested for the presence of RSV, adenovirus, influenza A and B virus, and parainfluenza virus type 1, 2 and 3 using the Virus Respiratory Kit (Bartels: Trinity Biotech plc, Wicklow Ireland). In addition, PCR tests for rhinoviruses, enterovirus, metapneumovirus and HCoV-OC43 and HCoV-229E were performed (2, 10). The original nasopharyngeal aspirate was inoculated onto a variety of cells. The cultures were kept in a roller drum at 34°C and observed every 3 to 4 days. Maintenance medium was replenished every 3 to 4 days. Two different types of medium were implemented: Optimem 1 (InVitrogen, Breda, The Netherlands) without bovine fetal serum was used for the tMK cells and MEM Hanks' /Earle's medium (InVitrogen, Breda, The Netherlands) with 3% bovine fetal serum was used for the remaining cell types. Cell cultures that were infected with the aspirate specimen were stained for the presence of respiratory viruses after one week of incubation. Direct staining was performed with pools of fluorescent-labeled mouse antibodies against RSV and influenza A and B virus (Imagen, DakoCytomation Ltd, Cambridge, UK). Indirect staining was performed for adenoviruses and parainfluenza virus type 1, 2 or 3 with mouse antibodies (Chemicon International, Temecula, California) and subsequent staining with FITC-labeled rabbit anti-mouse antibodies (Imagen, DakoCytomation Ltd, Cambridge, UK).

VIDISCA method

[0060] To remove residual cells and mitochondria, 110 µl of virus culture supernatant was spun down for 10 min at maximum speed in an eppendorf microcentrifuge (13500 rpm). To remove chromosomal DNA and mitochondrial DNA from the lysed cells, 100 µl was transferred to a fresh tube and treated with DNAse I for 45 min at 37°C (Ambion, Huntingdon, UK). Nucleic acids were extracted as described by Boom *et al*. (4). A reverse transcription reaction was performed with random hexamer primers (Amersham Bioscience, Roosendaal, The Netherlands) and MMLV-RT (InVitrogen, Breda The Netherlands) while second strand DNA synthesis was carried out with Sequenase II (Amersham Bioscience, Roosendaal, The Netherlands). A phenol/chloroform extraction was followed by an ethanol precipitation. The cDNA-AFLP was performed essentially as described by Bachem *et al* (1) with some modifications. The dsDNA was digested with the HinP I and Mse I restriction enzymes (New England Biolabs, Beverly, Massachusetts). Mse I- and HinP I-anchors (see below) were subsequently added with 5U ligase enzyme (InVitrogen, Breda, The Netherlands) in the supplied ligase buffer for 2 hrs at 37°C. The Mse I-and HinP I-anchors were prepared by mixing a top strand oligo (5'-CTCGTAGACTGCGTACC-3' for the Mse I anchor and 5'-GACGATGAGTCCTGAC-3' for the HinP I anchor) with a bottom strand oligo (5'-TAGGTACGCAGTC-3' for the Mse I anchor and 5'- CGGTCAGGACTCAT-3' for the HinP I anchor) in a 1:40 dilution of ligase buffer. A 20 cycle PCR was performed with 10 µl of the ligation mixture, 100 ng HinP I standard primer (5'-GACGATGAGTCCTGACCGC-3') and 100 ng Mse I standard primer (5'-CTCGTAGACTGCGTACCTAA-3'). Five µl of this PCR product was used as input in the second "selective" amplification step with 100 ng HinPI-N primer and 100 ng MseI-N (the "N" denotes that the standard primers are extended with one nucleotide: G, A, T or C). The selective rounds of amplification were done with a "touch down PCR": 10 cycles of [60 sec 94°C-30 sec 65°C-1 min 72°C] and the annealing temperature was reduced with 1 °C each cycle, followed by 23 cycles: [30 sec 94°C-30 sec 56 °C-1 min 72 °C] and 1 cycle 10 min 72°C. The PCR products were analyzed on 4% Metaphor® agarose gels (Cambrex, Rockland, Maine) and the fragments of interest were cloned and sequenced using BigDye terminator reagents. Electrophoresis and data collection was performed on an ABI 377 instrument.

cDNA library construction and full genome sequencing

[0061] The cDNA library was produced as described by Marra et al [17], with minor modifications. During reverse transcription only random hexamer primers were used and no oligo-dT primer, and the amplified cDNA was cloned into PCR2.1-TOPO TA cloning vector. Colonies were picked and suspended in BHI media. The E.coli suspension was used as input in a PCR amplification using T7 and M13 RP for amplification. The PCR products were subsequently sequenced with the same primers that were used in the PCR-amplification and the BigDye terminator reagent. Electrophoresis and data collection was performed on an ABI 377 instrument. Sequences were assembled using the AutoAssembler DNA sequence Assembly software version 2.0.

Diagnostic RT-PCR

[0062] From 492 persons a total of 600 respiratory samples collected between December 2002 and August 2002. The kind of material ranged from oral/nasopharyngeal aspirate, throat swabs, bronchioalveolary lavages and sputum.

The samples were collected for routine virus diagnostic screening of persons suffering from upper and lower respiratory tract disease. One hundred µl of the sample was used in a Boom extraction (4). The reverse transcription was performed with MMLV-RT (InVitrogen) using 10 ng or reverse transcription primer (repSZ-RT: 5'- CCACTATAAC-3'). The entire RT mixture was added to the first PCR mixture containing 100 ng of primer repSZ-1 (5'-GTGATGCATATGCTAATTTG-3') and 100 ng of primer repSZ-3 (5'-CTCTTGCAGGTATAATCCTA-3'). The PCR reaction was performed according to the profile 5min 95C; 20 cycles of: 1min 95°C-1min 55°C-2min 72°C; 10 min 72 °C. A nested PCR was started using 5 µl of the first PCR with 100 ng of primer repSZ-2 (5'-TTGGTAAACAAAAGATAACT-3') and 100 ng of primer repSZ-4 (5'-TCAATGCTATAAACAGTCAT-3'). Twenty-five PCR cycles were performed of the same profile as the first PCR. Ten µl of the PCR products was analyzed by agarose gel electrophoresis. All positive samples were sequenced to confirm the presence of HCoV-NL63 in the sample.

Sequence analysis

[0063] Sequences were compared to all sequences in the Genbank database using the BLAST tool of the NCBI web page: http://www.ncbi.nlm.nih.gov/blast. For phylogenetic analysis the sequences were aligned using the ClustalX software package with the following settings: Gap opening penalties: 10.00; Gap extension penalty 0.20; Delay divergent sequences switch at 30% and transition weight 0.5 (9). Phylogenetic analysis was carried out using the neighbor-joining method of the MEGA program (5) using the information of all fragments within one gene. The nucleotide distance matrix was generated either by Kimura's 2 parameter estimation or by the p-distance estimation (6). Bootstrap resampling (500 replicates) was employed to place approximate confidence limits on individual branches.

Results

Virus isolation from a child with acute respiratory disease

[0064] In January 2003 a 7-month-old child appeared in the hospital with coryza, conjunctivitis and fever. Chest radiography showed typical features of bronchiolitis and a nasopharyngeal aspirate specimen was collected five days after the onset of disease (sample NL63). Diagnostic tests for respiratory syncytial virus (RSV), adenovirus, influenza A and B virus, parainfluenza virus type 1, 2 and 3, rhinovirus, enterovirus, HCoV-229E and HCoV-OC43 remained negative. The clinical sample was subsequently inoculated onto human fetal lung fibroblasts (HFL), tertiary monkey kidney cells (tMK; Cynomolgus monkey) and HeLa cells. CPE was detected exclusively on tMK cells and first noted at eight days post-inoculation. The CPE was diffuse with a refractive appearance in the affected cells followed by cell detachment after 7 days. More pronounced CPE was observed upon passage onto the monkey kidney cell line LLC-MK2 with overall cell rounding and moderate cell enlargement (Fig. 1). Additional subcultures on HFL, rhabdomyosarcoma cells and Vero cells remained negative for CPE. Immunofluorescent assays to detect RSV, adenovirus, influenza A and B virus, or parainfluenza virus type 1, 2 and 3 in the culture remained negative. Acid lability and chloroform sensitivity tests demonstrated that the virus is most likely enveloped and not a member of the picornavirus group[24].

Virus discovery by the VIDISCA method

[0065] Identification of unknown pathogens by molecular biology tools encounters the problem that the target sequence is not known and that genome specific PCR-primers cannot be designed. To overcome this problem we developed the VIDISCA method that is based on the cDNA-AFLP technique[4]. The advantage of VIDISCA is that prior knowledge of the sequence is not required as the presence of restriction enzyme sites is sufficient to guarantee amplification. The input sample can be either blood plasma/serum or culture supernatant. Whereas cDNA-AFLP starts with isolated mRNA, the VIDISCA technique begins with a treatment to selectively enrich for viral nucleic acid, which includes a centrifugation step to remove residual cells and mitochondria. In addition, a DNAse treatment is used to remove interfering chromosomal DNA and mitochondrial DNA from degraded cells, whereas viral nucleic acid is protected within the viral particle. Finally, by choosing frequently cutting restriction enzymes, the method is fine-tuned such that most viruses will be amplified. Using VIDISCA we were able to amplify viral nucleic acids from EDTA-plasma of a person with hepatitis B virus infection and a person with an acute parvovirus B19 infection. The technique can also detect HIV-1 in cell culture, demonstrating its capacity to identify both RNA and DNA viruses.

[0066] The supernatant of the CPE-positive culture NL63 was analyzed by VIDISCA. We used the supernatant of uninfected cells as a control. After the second PCR amplification step, unique and prominent DNA fragments were present in the test sample but not in the control. These fragments were cloned and sequenced. Twelve out of 16 fragments showed sequence similarity to members of the family of coronaviruses, but significant sequence divergence was apparent in all fragments. These results indicate that we identified a novel coronavirus (HCoV-NL63).

Detection of HCoV-NL63 in patient specimens

[0067] To demonstrate that HCoV-NL63 originated from the nasopharyngeal aspirate of the child, we designed a diagnostic RT-PCR that specifically detects HCoV-NL63. This test, based on unique sequences within the 1b gene, confirmed the presence of HCoV-NL63 in the clinical sample. The sequence of this PCR product was identical to that of the virus identified upon in vitro passage in LLC-MK2 cells (results not shown).

[0068] Having confirmed that the cultured coronavirus originated from the child, the question remains whether this is an isolated clinical case or whether HCoV-NL63 is circulating in humans. To address this question, we examined respiratory specimens of hospitalized persons and individuals visiting the outpatient clinic between December 2002 and August 2003 for the presence of HCoV-NL63. We identified 7 additional persons that carried HCoV-NL63 . Sequence analysis of the PCR products indicated the presence of a few characteristic (and reproducible) point mutations in several samples, suggesting that several subgroups of NL63 may co-circulate. At least 5 of the HCoV-NL63-positive individuals suffered from a respiratory tract illness, the clinical data of 2 persons were not available. Including the index case, five patients were children less than 1 year old and 3 patients were adults. Two adults are likely to be immunosuppressed, as one of them is a bone marrow transplant recipient, and the other is an HIV positive patient suffering from AIDS with very low CD4 cell counts. No clinical data of the third adult was available. Only 1 patient had a co-infection with RSV (nr 72), and the HIV-infected patient (nr 466) carried *Pneumocystis carinii.* No other respiratory agent was found in the other HCoV-NL63-positive patients, suggesting that the respiratory symptoms were caused by HCoV-NL63. All HCoV-NL63 positive samples were collected during the last winter season, with a detection frequency of 7% in January 2003. None of the 306 samples collected in the spring and summer of 2003 contained the virus ($P < 0.01$, 2-tailed t-test).

Complete genome analysis of HCoV-NL63

[0069] The genomes of coronaviruses have a characteristic, genome organization. The 5' half contains the large 1a and 1b genes, encoding the non-structural polyproteins, followed by the genes coding for four structural proteins: spike (S), membrane (M), envelope (E) and the nucleocapsid (N) protein. Additional non-structural proteins are encoded either between 1b and the S gene, between the S and E gene, between the M and N gene or downstream of the N gene.

[0070] To determine whether the HCoV-NL63 genome organization shares these characteristics, we constructed a cDNA library with a purified virus stock as input material. A total of 475 genome fragments were analyzed, with an average coverage of 7 sequences per nucleotide. Specific PCRs were designed to fill in gaps and to sequence regions with low quality sequence data. Combined with 5'RACE (Rapid Amplification of cDNA Ends) and 3'RACE experiments the complete HCoV-NL63 genome sequence was resolved.

[0071] The genome of HCoV-NL63 is a 27,553-nucleotide RNA with a poly A tail. With a G-C content of 34% it has the lowest G-C content among the *coronaviridae*, which range from 37%-42%[25]. ZCurve software was used to identify ORFs[26] and the genome configuration is portrayed using the similarity with known coronaviruses (Fig. 6). The 1a and 1b genes encode the RNA polymerase and proteases that are essential for virus replication. A potential pseudoknot structure is present at position 12439, which may provide the -1 frameshift signal to translate the 1b polyprotein. Genes predicted to encode the S, E, M and N proteins are found in the 3' part of the genome. Short untranslated regions (UTRs) of 286 and 287 nucleotides are present at the 5' and 3' termini, respectively. The hemagglutinin-esterase gene, which is present in some group 2 and group 3 coronaviruses, was not present. ORF 3 between the S and E gene probably encodes a single accessory non-structural protein.

[0072] The 1a and 1ab polyproteins are translated from the genomic RNA, but the remaining viral proteins are translated from subgenomic mRNAs (sg mRNA), each with a common 5' end derived from the 5' part of the genome (the 5' leader sequence) and 3' coterminal parts. The sg mRNA are made by discontinuous transcription during negative strand synthesis[27]. Discontinuous transcription requires base-pairing between *cis*-acting transcription regulatory sequences (TRSs), one located near the 5' part of the genome (the leader TRS) and others located upstream of the respective ORFs (the body TRSs)[28]. The cDNA bank that we used for sequencing contained copies of sg mRNA of the N protein, thus providing the opportunity to exactly map the leader sequence that is fused to all sg mRNAs. A leader of 72 nucleotides was identified at the 5' UTR. The leader TRS (5'-UCUCAACUAAAC-3') showed 11/12-nucleotide similarity with the body TRS upstream of the N gene. A putative TRS was also identified upstream of the S, ORF 3, E and M gene .

[0073] The sequence of HCoV-NL63 was aligned with the complete genomes of other coronaviruses. The percentage nucleotide identity was determined for each gene. For all genes except the M gene, the percentage identity was the highest with HCoV-229E. To confirm that HCoV-NL63 is a new member of the group 1 coronaviruses, phylogenetic analysis was performed using the nucleotide sequence of the 1A, 1B, S, M and N gene. For each gene analyzed, HCoV-NL63 clustered with the group 1 coronaviruses. The bootstrap values of the subgroup HCoV-NL63/HCoV-229E were 100 for the 1a, 1b and S gene. However, for the M and N gene the bootstrap values of this subcluster decreased

(to 78 and 41 respectively) and a subcluster containing HCoV-229E, HCoV-NL63 and PEDV becomes apparent. A phylogenetic analysis could not be performed for the ORF 3 and E gene because the region varied too much between the different coronavirus groups or because the region was too small for analysis, respectively. Bootscan analysis by the Simplot software version 2.5 [29] found no signs of recombination (results not shown)..

[0074] The presence of a single non-structural protein gene between the S and E gene is noteworthy since almost all coronaviruses have 2 or more ORFs in this region, with the exception of PEDV and OC43 [30,31]. Perhaps most remarkable is a large insert of 537 nucleotides in the 5'part of the S gene when compared to HCoV-229E. A Blast search found no similarity of this additional 179-amino acid domain of the spike protein to any coronavirus sequence or any other sequences deposited in GenBank.

Tables

[0075]

Table 1: cDNA- AFLP oligonucleotides for virus discovery

Oligo	Sequence
Top strand MSE adaptor	CTCGTAGACTGCGTACC
Top strand for HinP1 adaptor	GACGATGAGTCCTGAC
Bottom strand oligo for MSE adaptor	TAGGTACGCAGTC
Bottom strand oligo for HinP1 adaptor	CGGTCAGGACTCAT
HinPI standard primer	GACGATGAGTCCTGACCGC
MseI standard primer	CTCGTAGACTGCGTACCTAA

Table 2: Oligonucleotide for PALM extension of the HCOV-NL63 Sequence

Oligonucleotide name,	Application,	Sequence 5'- 3'
JZH2R	1st PCR	GCTATCATCACAATGGACNNNNNG

Table 3. Nucleotide- and corresponding deduced amino acid sequences

Fragment	Sequence
163-2	GTATTGTTTTTGTTGCTTGTGCCCATGCTGCTGTTGATTCCTTATGTGCAAAAGCTATGA CTGTTTATAGCATTGATAAGTGTACTAGGATTATACCTGCAAGAGCTCGGGTTGAGTGTT ATAGTGGCT
163-2 Translation	Replicase polyprotein 1a IVFVACAHAAVDSLCAKAMTVYSIDKCTRIIPARARVECYSG
163-4	ATGGGTCTAGATATGGCTTGCAAAACTTACTACAGTTACCTAACTTTTATTATGTTAGTA ATGGTGGTAACAATTGCACTACGGCCGTTATGACCTATTCTAATTTTGGTATTTGTGCTG ATGGTTCTTTGATTCCTGTTCGTCC
163-4 Translation	Spike protein GSRYGLQNLLQLPNFYYVSNGGNNCTTAVMTYSNFGICADGSLIPVR
163-9 (3'-UTR)	ATGATAAGGGTTTAGTCTTACACACAATGGTAGGCCAGTGATAGTAAAGTGTAAGTAATT TGCTATCATAT
163-10	ATGTCAGTGATGCATATGCTAATTTGGTTCCATATTACCAACTTATTGGTAAACAAAAGA TAACTACAATACAGGGTCCTCCTGGTAGTGGTAAGTCACATTGTTCCATTGGACTTGGAT TGTACTACCCAGGT
163-10 Translation	Replicase polyprotein 1ab VSDAYANLVPYYQLIGKQKITTIQGPPGSGKSHCSIGLGLYYPG
163-11	ATCTAAACTAAACAAAATGGCTAGTGTAAATTGGGCCGATGACAGAGCTGCTAGGAAGAA ATTTCCTCCTCCTTCATTTTACATGCCTCTTTTGGTTAGTTCTGATAAGGCACCATATAG GGTCATTCCCAGGAATCTTGTCCCTATTGGTAAGGGTAATAAAGATGAGCAGATTGGTTA TTGGAATGTTCAAGAGCGTTGGCGTAT
163-11 Translation	Nucleocapsid protein SKLNKMASVNWADDRAARKKFPPPSFYMPLLVSSDKAPYRVIPRNLVPIGKGNKDEQIGY WNVQERWR
163-14	ACAAAAATTTGAATGAGGGTGTTCTTGAATCTTTTTCTGTTACACTTCTTGATAATCAAG AAGATAAGTTTTGGTGTGAAGATTTTTATGCTAGTATGTATGAAAATTCTACAATATTGC AAGCTGCTGGTTTATGTGTTGTTTGTGGTTCACAAACTGTACTTCGTTGTGGTGATTGTC TGCGTAAGCCTATGTTGTGCACTAAAT
163-14 Translation	Replicase polyprotein 1ab KNLNEGVLESFSVTLLDNQEDKFWCEDFYASMYENSTILQAAGLCVVCGSQTVLRCGDCL RKPMLCTK
163-15	AGGGGGCAACGTGTTGATTTGCCTCCTAAAGTTCATTTTTATTACCTAGGTACTGGACCT CATAAGGACCT

163-15 Translation	Nucleocapsid protein RGQRVDLPPKVHFYYLGTGPHKD
163-18	TAGTAGTTGTGTTACTCGTTGTAATATAGGTGGTGCTGTTTGTTCAAAACATGCAAATTT GTATCAAAAATACGTTGAGGCATATAATACATTTACACAGGCAGGTT
163-18 Translation	Replicase polyprotein 1ab SSCVTRCNIGGAVCSKHANLYQKYVEAYNTFTQAG

Table 4:

Identification of cDNA-AFLP fragments	
Fragment	Identification best Blast hit
163-2	replicase polyprotein 1ab [Human coronavirus 229E]
163-4	spike protein [Human coronavirus 229E]
163-9	3'UTR Human coronavirus 229E
163-10	replicase polyprotein 1ab [Human coronavirus 229E]
163-11	replicase polyprotein 1ab [Human coronavirus 229E]
163- 14	replicase polyprotein 1ab [Human coronavirus 229E]
163-15	nucleocapsid protein [Human coronavirus 229E]
163-18	replicase polyprotein 1ab [Human coronavirus 229E]

Table 5:

Pairwise nucleotide sequence homologies between the virus of the present invention and different corona (like) viruses in percentages sequence identity (%)							
Fragment	BcoV	MHV	HcoV	PEDV	TGE	SARS	IBV
Replicase 1AB 163-2	59.6	61.2	76.7	70.5	64.3	65.8	64.3
Spike gene 163-4	31.7	26.5	64.6	48.9	45.4	33.7	25.9
3'UTR 163-9	29.5	34	81.9	53.6	50	31.5	38
Replicase 1AB 163-10	55.2	57.4	82	73.8	69.4	64.1	65.1
Nucleocapsid 163-11	25.5	23.8	54.9	51.5	44.6	23.3	27.6
Replicase 1AB 163-14	52.1	52.1	78.7	72.9	76.3	52.6	58.4
Nucleocapsid 163-15	29.5	35.2	71.8	63.3	60.5	25.3	45
Replicase 1AB 163-18	67.2	65.4	72.8	65.4	61.6	68.2	57

Table 6:

Pairwise deduced amino acid sequence homologies between different corona (like) viruses in percentages sequence identity (%)							
Fragment	BCoV	MHV	HcoV	PEDV	TGE	SARS	IBV
Replicase 1AB 163-2	55.8	53.4	88.3	79	60.4	67.4	55.8
Spike gene 163-4	ND	ND	56.2	ND	ND	ND	ND
Replicase 1AB 163-10	51.1	53.3	93.3	86.6	80	57.7	55.5
Nucleocapsid 163-11	ND	ND	48.4	ND	ND	ND	ND
Replicase 1AB 163-14	50.7	50.7	86.9	78.2	78.2	46.3	47.8
Nucleocapsid 163-15	ND	ND	82.6	ND	ND	ND	ND
Nucleocapsid 163-18	63.8	63.8	77.7	69.4	69.4	58.3	55.5
ND = Not Determined							

Table 7: Oligos for specific detection of HcoV-163

Primer	Sequence
repSZ-RT	CCACTATAAC
repSZ-1	GTGATGCATATGCTAATTTG
repSZ-2	TTGGTAAACAAAAGATAACT
repSZ-3	CTCTTGCAGGTATAATCCTA
repSZ-4	TCAATGCTATAAACAGTCAT

Table 8:

Molecule Features			
Start	End	Name	Description
287	12439	1a	ORF-1a
4081	4459		Pfam 01661
9104	10012		3Cl protease
12433	12439		Ribosome slippery site
12439	20475	1b	ORF-1b
14166	14490		Pfam 00680
16162	16965		COG1112, Super family DNA and RNA helicase
16237	16914		Pfam 01443 Viral helicase
20472	24542	2	ORF-2 S(pike)-gene
21099	22619		S1 Pfam 01601
22625	24539		S2 Pfam 01601

Table 8: (continued)

Molecule Features			
Start	End	Name	Description
24542	25219	3	ORF-3
24551	25174		NS3b Pfam 03053
25200	25433	4	ORF-4 Pfam 05780, Coronavirus NS4 E (envelope) protein
25442	26122	5	ORF-5
25442	26119		Matrix glycoprotein Pfam 01635 M-gene
26133	27266	6	ORF-6
26184	27256		Nucleocapsid Pfam 00937 N-gene
Via a -1 frame shift at the ribosome slippery site the 1a ORF is extended to protein of 6729 amino acid residues referred to as 1ab. ORF 1a and 1ab encode two polyproteins that are proteolytically converted to 16 largely uncharacterized enzymes that are involved in RNA replication (for review see Snijder, E. J., P. J. Bredenbeek, J. C. Dobbe, V. Thiel, J. Ziebuhr, L. L. Poon, Y. Guan, M. Rozanov, W. J. Spaan, and A. E. Gorbalenya. 2003. Unique and Conserved Features of Genome and Proteome of SARS-coronavirus, an Early Split-off From the Coronavirus Group 2 Lineage. J. Mol. Biol. **331**:991-1004).			

Table 9:

Proteins from HcoV-NL63 ORFs			
ORF	Number of AA	M_w prediction	
1a	4060	451364	Polyprotein
1ab	6729	752822	Polyprotein
2	1356	149841	Spike
3	225	25658	
4	77	9177	Envelope
5	226	25927	Matrix
6	377	42252	Nucleocapsid

[0076] The M_w prediction does not take into account post-translational modification like glycosylation or cleavage of a signal sequence.

Table 10: Amplification oligonucleotides for HCoV-NL65 S, M and N encoding regions

Primer	Sequence
S1	ACAAGTTTGTACAAAAAAGCAGGCTTCAAACTTTTCTTGATTTTGCTTGTTTTGCCCC
S2	ACCACTTTGTACAAGAAAGCTGGGTCTTGAACGTGGACCTTTTCAAATTCG
M1	ACAAGTTTGTACAAAAAAGCAGGCTTCTCTAATAGTAGTGTGCCTCTTTTAGAGG
M2	ACCACTTTGTACAAGAAAGCTGGGTCGATTAAATGAAGCAACTTCTC
N1	ACAAGTTTGTACAAAAAAGCAGGCTTCGCTAGTGTAAATTGGGCCGATG
N2	ACCACTTTGTACAAGAAAGCTGGGTCATGCAAAACCTCGTTGACAATTTCTATAATGGC

[0077] The S, M and N complementary sequences are indicated in bold print. The remainder of the PCR primers is composed of either in-frame *att*B1 or *att*B2 sites

Table 11:

Overall full length genome DNA sequence identity							
	BCV	**HC229 E**	**IBV**	**SARS**	**TGV**	**HCoV- NL63**	**HCoV- OC43**
BCV	100	46	43	54	40	43	95
HC229 E		100	50	48	53	65	46
IBV			100	43	46	48	43
SARS				100	40	43	53
TGV					100	55	40
HCoV-NL63						100	43
OC43							100

[0078] Overall DNA sequence identity percentages of HCoV-NL63 compared to other coronaviruses. From the Sim-Plot graph (Fig. 7), comparing HCoV-NL63 (query) with SARS associated coronavirus and HCoV-229E, can be deduced that local sequence identity never exceeds 85%

Table 12:

Overall DNA sequence identity Spike encoding region				
	OC43	NL63	229E	SARS
OC43	100	46	40	44
NL63		100	59	38
229E			100	41
SARS				100

Table 13:

Overall DNA sequence identity in 5'UTR				
	OC43	NL63	229E	SARS
OC43	100	36	34	48
NL63		100	74	33
229E			100	34
SARS				100

Brief description of the drawings

[0079]

Figure 1
cDNA-AFLP allows amplification of nucleic acids without any prior sequence information.
Culture supernatants from CPE-positive and uninfected cells are subjected to the cDNA-AFLP procedure. Amplification products derived from the CPE-positive culture which are not present in the uninfected control sample are cloned and sequenced.

Figure 2
LLC-MK2 cells infected with HCoV-NL163.
Panel A and B are unstained cells while panel C and D are stained with haematoxilin eosin. The typical CPE of HCoV-NL163 is shown in panel A and C. The control uninfected LLC-MK cells are shown in panel B and D.

Figure 3
VD-cDNA-AFLP PCR products visualized by Metaphor® agarose gel electrophoreses.
The PCR products of 1 (HinP I-G and Mse I-A) of 16 primer pair combinations used during the selective amplification step. Lanes 1 and 2: duplicate PCR product of virus culture NL163; lanes 5 and 6 control supernatant of LLC-MK2 cells and in lane 7 and 8 the negative PCR control. Lanes M: 25bp molecular weight marker (InVitrogen). The arrow indicates a new coronavirus fragment that was excised out of gel and sequenced.

Figure 4
Phylogenetic analysis of the HCoV-163 sequences.
G1, G2 and G3 denote the group 1, group 2 and group 3 coronavirus clusters.
The Genbank accession number of the used sequences are: MHV (mouse hepatitis virus): AF201929; HCoV-229E: AF304460; PEDV (porcine epidemic diarrhea virus): AF353511; TGEV (transmissible gastroenteritis virus): AJ271965; SARS-CoV: AY278554; IBV (avian infectious bronchitis virus): NC_001451; BCoV (bovine coronavirus): NC_003045; FCoV (feline coronavirus): Y13921 and X80799; CCoV (canine coronavirus): AB105373 and A22732; PRCoV (porcine respiratory coronavirus) : M94097; FIPV (feline infectious peritonitis virus): D32044. Position of the HCoV-163 fragments compared to HCoV-229E (AF304460): Replicase 1AB gene: 15155-15361, 16049-16182, 16190-16315, 18444-18550, Spike gene: 22124-22266; Nucleocapsid gene: 25667-25882 and 25887-25957; 3'UTR: 27052-27123. Branch lengths indicate the number of substitutions per sequence.

Figure 5
Schematic representation of Coronavirus and the location of the 163-fragments listed in table 3.

Figure 6
Restriction map of HCoV-NL63'
Complete 27553 nt cDNA derivative of the ssRNA genome. Open reading frames (ORF) are depicted as numbered black arrows and the identified (PFAM) domains within these ORFs are indicated as gray boxes.

Figure 7
Simplot analysis HcoV NL63 and other human Coronaviruses
The gap in the comparison of HCoV NL63 to SARS, HCoV-OC43 and HCoV-229E is cause by a unique 537 in-frame insertion in the Spike protein encoding ORF (see elsewhere herein). Sigmaplot analysis is described in Lole, K. S., R. C. Bollinger, R. S. Paranjape, D. Gadkari, S. S. Kulkarni, N. G. Novak, R. Ingersoll, H. W. Sheppard, and S. C. Ray. 1999. Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. J. Virol. 73:152-160.

Figure 8
Expression constructs for HCoV-NL63 Spike and Matrix protein
Expression of a His and StrepII tagged Spike fusion protein can be induced by addition of IPTG to the bacterial growth medium. Through attB1/B2-mediated recombination, the S gene insert can be transferred to other commercially available expression vectors, facilitating protein production in other hosts.
Through an identical cloning procedure as for pGP7S, a Gateway compatible expression vector for HCoV-NL63 M-gene can be constructed. The plasmid directs IPTG inducible production of N and C-terminally affinity tagged Matrix fusion protein, allowing selective recovery of full-length fusion protein.

Figure 9
Recombination site NL63-229E
NL63-derived sequences are in underlined bold black print and the 229E derived sequences are in gray bold print.

Figure 10
Restriction map cDNA Clone NL63/229E hybrid
The NL63 derived part is indicated as gray boxes and the 229E-derived region is indicated as a line. The junction between the two genomes is indicated by the succession of the two black arrows marked 1b' and 'ORF-1b indicating the hybrid 1b ORF.
A second chimeric genome was generated by a reciprocal recombination fusing nucleotide 19653 of HCoV-NL63 to nucleotide 20682 of HCoV-OC43 again creating a hybrid ORF 1b giving rise to a hybrid 1ab replicase polyprotein. Recombination occurred within the conserved sequence AATTATGG

Figure 11
Recombination site NL63/OC43 hybrid.
Again, NL63-derived region is in bold black underlined print and the OC43 derived sequences are in gray bold print. The resulting cDNA restriction map is depicted in Figure 12

Figure 12
Restriction map recombinant NL63/OC43 genome.
The NL63-derived part is indicated as gray boxes and the recombination site is depicted as the between the black arrows 1b' and '1b.

Figure 13
Similarity plot deduced protein alignments of ORF1b from HCoV-NL63,
HCoV-229E, HCoV-OC43 and the two hybrids NL63/229E and NL63/OC43.

Figure 14
Green fluorescent protein expressing HcoV-NL63 derivative.
Functional equivalent NL63/4GFP carries an in-frame C-terminal fusion of the E protein (ORF4) with a human codon optimised Green Fluorescent Protein (EGFP, Stratagene). Infected cells appear fluorescent after excitation of the 4-EGFP fusion protein. HCoV-NL63 can be used to elucidate the process of viral; infection and the translation of the polycistronic sub-genomic messengers.

Figure 15
Restriction map of functional derivative NL63D2052021011.
This deletion derivative of NL63 lacks most of the insertion at the N-terminal end of the Spike protein. By deleting nucleotides 20520-21011 the unique domain is removed while retaining the predicted secretory signal sequence (Nielsen, H., J. Engelbrecht, S. Brunak, and G. Von Heijne. 1997. Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. Protein Eng 10:1-6).

Figure 16
Sequence variation in HCoV-NL63 from additional patient samples
Direct sequencing of both strands of RT-PCR products from 6 patient samples revealed the presence of polymorphisms in the ORF 1a region.

Figure 17
HCoV-NL63 specific and generic human Coronavirus detection probes. Coronavirus polymerases generate several sub-genomic RNAs. The frequency of S, E, M and N protein encoding cDNA clones in the sequencing library of HCoV-NL63 and SARS (Snijder, E. J., P. J. Bredenbeek, J. C. Dobbe, V. Thiel, J. Ziebuhr, L. L. Poon, Y. Guan, M. Rozanov, W. J. Spaan, and A. E. Gorbalenya. 2003). Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage. J. Mol. Biol. 331:991-1004). Northern blot data demonstrate a high abundance of these sub-genomic RNAs in infected cells. Consequently, these genes are attractive targets for diagnostic tests.
Since the genomic and sub-genomic RNAs possess identical 3'ends, probes containing the N gene would hybridise to all of them (Table 8).
Through alignment of the full-length sequences of all human Coronaviruses a conserved region in ORF1b was identified, allowing their detection with a nested RT-PCR assay.

Figure 18
Generic Coronavirus detection primers

Figure 19
Nucleotide sequence an HcoV_NL63

Figure 20
ORF 1a, replicase enzyme complex of an HcoV_NL63

Figure 21
ORF 1ab replicase polyprotein of an HcoV_NL63

Figure 22
The spike protein (ORF3) contains an N-terminal secretory signal sequence of 16 AA (indicated on the first line of the continuous sequence listed below). (Nielsen, H., J. Engelbrecht, S. Brunak, and G. Von Heijne. 1997. Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. Protein Eng 10:1-6)

Figure 23
ORF-4 Coronavirus_NS4, Coronavirus non-structural protein 4. This family consists of several non-structural protein 4 (NS4) sequences or small membrane protein.
ORF-5. This family consists of various coronavirus matrix proteins that are transmembrane glycoproteins. The M protein or E1 glycoprotein is implicated in virus assembly. The E1 viral membrane protein is required for formation of the viral envelope and is transported via the Golgi complex. The matrix protein is predicted to contain an N-terminal secretory signal sequence (indicated in the first part of the continuous sequence) (Nielsen, H., J. Engelbrecht, S. Brunak, and G. Von Heijne. 1997. Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. Protein Eng 10:1-6.)
ORF-6 Pfam 00937, Coronavirus nucleocapsid protein. Structural protein forming complexes with the genomic RNA

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<213> Human Coronavirus 229E

<400> 26

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Tyr Glu Asn Ser Thr Ile Leu Gln Ala Ala Gly Leu Cys Val Val Cys
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<212> DNA
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<213> Human Coronavirus 229E

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<210> 30
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<213> Human Coronavirus 229E

<400> 30

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<223> Primer M1

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<220>
<223> Conserved sequence

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<211> 69
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<210> 39
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<223> Sequence 223B

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<223> "n" stands for "i"

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Leu Gln Asp Cys Val Thr Gly Ile Asn Asp Asp Asp Tyr Val Ile Ala
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Leu Thr Gly Thr Asn Gln Leu Cys Ala Lys Ile Leu Pro Phe Ser Asp
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Arg Pro Leu Asn Leu Arg Gly Trp Leu Ile Phe Ser Asn Ser Asn Tyr
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Val Leu Gln Asp Phe Asp Val Val Phe Gly His Gly Ala Gly Ser Val

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Val Phe Val Asp Lys Tyr Met Cys Gly Phe Asp Gly Lys Pro Val Leu
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Pro Lys Asn Met Trp Glu Phe Arg Asp Tyr Phe Asn Asn Asn Thr Asp
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Ser Ile Val Ile Gly Gly Val Thr Tyr Gln Leu Ala Trp Asp Val Ile
145                 150                 155                 160

Arg Lys Asp Leu Ser Tyr Glu Gln Gln Asn Val Leu Ala Ile Glu Ser
                165                 170                 175

Ile His Tyr Leu Gly Thr Thr Gly His Thr Leu Lys Ser Gly Cys Lys
            180                 185                 190

Leu Thr Asn Ala Lys Pro Pro Lys Tyr Ser Ser Lys Val Val Leu Ser
        195                 200                 205

Gly Glu Trp Asn Ala Val Tyr Arg Ala Phe Gly Ser Pro Phe Ile Thr
    210                 215                 220

Asn Gly Met Ser Leu Leu Asp Ile Ile Val Lys Pro Val Phe Phe Asn
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Ala Phe Val Lys Cys Asn Cys Gly Ser Glu Ser Trp Ser Val Gly Ala
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Trp Asp Gly Tyr Leu Ser Ser Cys Cys Gly Thr Pro Ala Lys Lys Leu
            260                 265                 270

Cys Val Val Pro Gly Asn Val Val Pro Gly Asp Val Ile Ile Thr Ser
        275                 280                 285

Thr Ser Ala Gly Cys Gly Val Lys Tyr Tyr Ala Gly Leu Val Val Lys
    290                 295                 300

His Ile Thr Asn Ile Thr Gly Val Ser Leu Trp Arg Val Thr Ala Val
305                 310                 315                 320

His Ser Asp Gly Met Phe Val Ala Ser Ser Ser Tyr Asp Ala Leu Leu
                325                 330                 335
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His Arg Asn Ser Leu Asp Pro Phe Cys Phe Asp Val Asn Thr Leu Leu
            340                 345                 350

Ser Asn Gln Leu Arg Leu Ala Phe Leu Gly Ala Ser Val Thr Glu Asp
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Val Lys Phe Ala Ala Ser Thr Gly Val Ile Asp Ile Ser Ala Gly Met
    370                 375                 380

Phe Gly Leu Tyr Asp Asp Ile Leu Thr Asn Asn Lys Pro Trp Phe Val
385                 390                 395                 400

Arg Lys Ala Ser Gly Leu Phe Asp Ala Ile Trp Asp Ala Phe Val Ala
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Ala Ile Lys Leu Val Pro Thr Thr Thr Gly Val Leu Val Arg Phe Val
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Lys Ser Ile Ala Ser Thr Val Leu Thr Val Ser Asn Gly Val Ile Ile
        435                 440                 445

Met Cys Ala Asp Val Pro Asp Ala Phe Gln Ser Val Tyr Arg Thr Phe
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Thr Gln Ala Ile Cys Ala Ala Phe Asp Phe Ser Leu Asp Val Phe Lys
465                 470                 475                 480

Ile Gly Asp Val Lys Phe Lys Arg Leu Gly Asp Tyr Val Leu Thr Glu
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Asn Ala Leu Val Arg Leu Thr Thr Glu Val Val Arg Gly Val Arg Asp
            500                 505                 510

Ala Arg Ile Lys Lys Ala Met Phe Thr Lys Val Val Val Gly Pro Thr
        515                 520                 525

Thr Glu Val Lys Phe Ser Val Ile Glu Leu Ala Thr Val Asn Leu Arg
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Leu Val Asp Cys Ala Pro Val Val Cys Pro Lys Gly Lys Ile Val Val
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Ile Ala Gly Gln Ala Phe Phe Tyr Ser Gly Gly Phe Tyr Arg Phe Met
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Val Asp Pro Thr Thr Val Leu Asn Asp Pro Val Phe Thr Gly Asp Leu
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Phe Tyr Thr Ile Lys Phe Ser Gly Phe Lys Leu Asp Gly Phe Asn His
        595                 600                 605

Gln Phe Val Thr Ala Ser Ser Ala Thr Asp Ala Ile Ile Ala Val Glu
    610                 615                 620

Leu Leu Leu Leu Asp Phe Lys Thr Ala Val Phe Val Tyr Thr Cys Val
625                 630                 635                 640

Val Asp Gly Cys Ser Val Ile Val Arg Arg Asp Ala Thr Phe Ala Thr
                645                 650                 655

His Val Cys Phe Lys Asp Cys Tyr Asn Val Trp Glu Gln Phe Cys Ile
            660                 665                 670

Asp Asn Cys Gly Glu Pro Trp Phe Leu Thr Asp Tyr Asn Ala Ile Leu
        675                 680                 685

Gln Ser Asn Asn Pro Gln Cys Ala Ile Val Gln Ala Ser Glu Ser Lys
    690                 695                 700

Val Leu Leu Glu Arg Phe Leu Pro Lys Cys Pro Glu Ile Leu Leu Ser
705                 710                 715                 720

Ile Asp Asp Gly His Leu Trp Asn Leu Phe Val Glu Lys Phe Asn Phe
                725                 730                 735

Val Thr Asp Trp Leu Lys Thr Leu Lys Leu Thr Leu Thr Ser Asn Gly
            740                 745                 750

Leu Leu Gly Asn Cys Ala Lys Arg Phe Arg Arg Val Leu Val Lys Leu
        755                 760                 765

Leu Asp Val Tyr Asn Gly Phe Leu Glu Thr Val Cys Ser Val Ala Tyr
    770                 775                 780
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Thr Ala Gly Val Cys Ile Lys Tyr Tyr Ala Val Asn Val Pro Tyr Val
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Val Ile Ser Gly Phe Val Ser Arg Val Ile Arg Arg Glu Arg Cys Asp
                805                 810                 815

Met Thr Phe Pro Cys Val Ser Cys Val Thr Phe Phe Tyr Glu Phe Leu
            820                 825                 830

Asp Thr Cys Phe Gly Val Ser Lys Pro Asn Ala Ile Asp Val Glu His
        835                 840                 845

Leu Glu Leu Lys Glu Thr Val Phe Val Glu Pro Lys Asp Gly Gly Gln
    850                 855                 860

Phe Phe Val Ser Gly Asp Tyr Leu Trp Tyr Val Val Asp Asp Ile Tyr
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Tyr Pro Ala Ser Cys Asn Gly Val Leu Pro Val Ala Phe Thr Lys Leu
                885                 890                 895

Ala Gly Gly Lys Ile Ser Phe Ser Asp Asp Val Ile Val His Asp Val
            900                 905                 910

Glu Pro Thr His Lys Val Lys Leu Ile Phe Glu Phe Glu Asp Asp Val
        915                 920                 925

Val Thr Ser Leu Cys Lys Lys Ser Phe Gly Lys Ser Ile Ile Tyr Thr
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Gly Asp Trp Glu Gly Leu His Glu Val Leu Thr Ser Ala Met Asn Val
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Ile Gly Gln His Ile Lys Leu Pro Gln Phe Tyr Ile Tyr Asp Glu Glu
                965                 970                 975

Gly Gly Tyr Asp Val Ser Lys Pro Val Met Ile Ser Gln Trp Pro Ile
            980                 985                 990

Ser Asn Asp Ser Asn Gly Cys Val  Val Glu Ala Ser Thr  Asp Phe His
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Gln Leu  Glu Cys Ile Val Asp  Asp Ser Val Arg Glu  Glu Val Asp
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Lys Gln  Pro Phe Ser Phe Ser  Phe Arg Asp Glu Leu  Gly Val Arg
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Val Leu  Asp Gln Ser Asp Asn  Asn Cys Trp Ile Ser  Thr Thr Leu
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Val Gln  Leu Gln Leu Thr Lys  Leu Leu Asp Asp Ser  Ile Glu Met
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Gln Leu  Phe Lys Val Gly Lys  Val Asp Ser Ile Val  Gln Lys Cys
1085                1090                1095

Tyr Glu  Leu Ser His Leu Ile  Ser Gly Ser Leu Gly  Asp Ser Gly
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Lys Leu  Leu Ser Glu Leu Leu  Lys Glu Lys Tyr Thr  Cys Ser Ile
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Thr Phe  Glu Met Ser Cys Asp  Cys Gly Lys Lys Phe  Asp Asp Gln
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Val Gly  Cys Leu Phe Trp Ile  Met Pro Tyr Thr Lys  Leu Phe Gln
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Lys Gly  Glu Cys Cys Ile Cys  His Lys Met Gln Thr  Tyr Lys Leu
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Val Ser  Met Lys Gly Thr Gly  Val Phe Val Gln Asp  Pro Ala Pro
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Ile Asp  Ile Asp Ala Phe Pro  Val Lys Pro Ile Cys  Ser Ser Val
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Tyr Leu  Gly Val Lys Gly Ser  Gly His Tyr Gln Thr  Asn Leu Tyr
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Ser Phe  Asn Lys Ala Ile Asp  Gly Phe Gly Val Phe  Asp Ile Lys
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Lys Asn  Val Lys Phe Tyr Leu  Gly Asp Ile Ser His  Leu Val Asn
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Cys Val  Ser Phe Asp Phe Val  Val Asn Ala Ala Asn  Glu Asn Leu
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Leu His  Gly Gly Gly Val Ala  Arg Ala Ile Asp Ile  Leu Thr Glu
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Gly Gln  Leu Gln Ser Leu Ser  Lys Asp Tyr Ile Ser  Ser Asn Gly
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Pro Leu  Lys Val Gly Ala Gly  Val Met Leu Glu Cys  Glu Lys Phe
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Asn Val  Phe Asn Val Val Gly  Pro Arg Thr Gly Lys  His Glu His
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Ser Leu  Leu Val Glu Ala Tyr  Asn Ser Ile Leu Phe  Glu Asn Gly
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Ile Pro  Leu Met Pro Leu Leu  Ser Cys Gly Ile Phe  Gly Val Arg
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Ile Glu  Asn Ser Leu Lys Ala  Leu Phe Ser Cys Asp  Ile Asn Lys
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Pro Leu  Gln Val Phe Val Tyr  Ser Ser Asn Glu Glu  Gln Ala Val
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Leu Lys  Phe Leu Asp Gly Leu  Asp Leu Thr Pro Val  Ile Asp Asp
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Val Asp  Val Val Lys Pro Phe  Arg Val Glu Gly Asn  Phe Ser Phe
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Asp Thr  Lys Leu Asn Gly Ile  Leu Gln Gln Ala Ala  Leu Asp Tyr
    1475                 1480                 1485

Leu Ala  Thr Val Lys Thr Val  Pro Ala Gly Asn Leu  Val Lys Leu
    1490                 1495                 1500

Phe Val  Glu Ser Cys Thr Ile  Tyr Met Cys Val Val  Pro Ser Ile
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Asn Asp  Leu Ser Phe Asp Lys  Asn Leu Gly Arg Cys  Val Arg Lys
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Leu Asn  Arg Leu Lys Thr Cys  Val Ile Ala Asn Val  Pro Ala Ile
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Asp Val  Leu Lys Lys Leu Leu  Ser Ser Leu Thr Leu  Thr Val Lys
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Phe Val  Val Glu Ser Asn Val  Met Asp Val Asn Asp  Cys Phe Lys
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Asn Asp  Asn Val Val Leu Lys  Ile Thr Glu Asp Gly  Ile Asn Val
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Lys Asp  Val Val Val Glu Ser  Ser Lys Ser Leu Gly  Lys Gln Leu
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Gly Val  Val Ser Asp Gly Val  Asp Ser Phe Glu Gly  Val Leu Pro
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Ile Asn  Thr Asp Thr Val Leu  Ser Val Ala Pro Glu  Val Asp Trp
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    1655                 1660                 1665

Arg Val  Leu Gly Thr Thr Asp  Asn Asn Cys Trp Val  Asn Ala Thr
    1670                 1675                 1680

Cys Ile  Ile Leu Gln Tyr Leu  Lys Pro Thr Phe Lys  Ser Lys Gly
    1685                 1690                 1695

Leu Asn  Val Leu Trp Asn Lys  Phe Val Thr Gly Asp  Val Gly Pro
    1700                 1705                 1710

Phe Val  Ser Phe Ile Tyr Phe  Ile Thr Met Ser Ser  Lys Gly Gln
    1715                 1720                 1725

Lys Gly  Asp Ala Glu Glu Ala  Leu Ser Lys Leu Ser  Glu Tyr Leu
    1730                 1735                 1740

Ile Ser  Asp Ser Ile Val Thr  Leu Glu Gln Tyr Ser  Thr Cys Asp
    1745                 1750                 1755

Ile Cys  Lys Ser Thr Val Val  Glu Val Lys Ser Ala  Ile Val Cys
    1760                 1765                 1770

Ala Ser  Val Leu Lys Asp Gly  Cys Asp Val Gly Phe  Cys Pro His
    1775                 1780                 1785

Arg His  Lys Leu Arg Ser Arg  Val Lys Phe Val Asn  Gly Arg Val
    1790                 1795                 1800

Val Ile  Thr Asn Val Gly Glu  Pro Ile Ile Ser Gln  Pro Ser Lys
    1805                 1810                 1815

Leu Leu  Asn Gly Ile Ala Tyr  Thr Thr Phe Ser Gly  Ser Phe Asp
    1820                 1825                 1830

Asn Gly  His Tyr Val Val Tyr  Asp Ala Ala Asn Asn  Ala Val Tyr
    1835                 1840                 1845

Asp Gly  Ala Arg Leu Phe Ser  Ser Asp Leu Ser Thr  Leu Ala Val
    1850                 1855                 1860

Thr Ala  Ile Val Val Val Gly  Gly Cys Val Thr Ser  Asn Val Pro
```

1865 1870 1875

Thr Ile Val Ser Glu Lys Ile Ser Val Met Asp Lys Leu Asp Thr
1880 1885 1890

Gly Ala Gln Lys Phe Phe Gln Phe Gly Asp Phe Val Met Asn Asn
1895 1900 1905

Ile Val Leu Phe Leu Thr Trp Leu Leu Ser Met Phe Ser Leu Leu
1910 1915 1920

Arg Thr Ser Ile Met Lys His Asp Ile Lys Val Ile Ala Lys Ala
1925 1930 1935

Pro Lys Arg Thr Gly Val Ile Leu Thr Arg Ser Phe Lys Tyr Asn
1940 1945 1950

Ile Arg Ser Ala Leu Phe Val Ile Lys Gln Lys Trp Cys Val Ile
1955 1960 1965

Val Thr Leu Phe Lys Phe Leu Leu Leu Leu Tyr Ala Ile Tyr Ala
1970 1975 1980

Leu Val Phe Met Ile Val Gln Phe Ser Pro Phe Asn Ser Leu Leu
1985 1990 1995

Cys Gly Asp Ile Val Ser Gly Tyr Glu Lys Ser Thr Phe Asn Lys
2000 2005 2010

Asp Ile Tyr Cys Gly Asn Ser Met Val Cys Lys Met Cys Leu Phe
2015 2020 2025

Ser Tyr Gln Glu Phe Asn Asp Leu Asp His Thr Ser Leu Val Trp
2030 2035 2040

Lys His Ile Arg Asp Pro Ile Leu Ile Ser Leu Gln Pro Phe Val
2045 2050 2055

Ile Leu Val Ile Leu Leu Ile Phe Gly Asn Met Tyr Leu Arg Phe
2060 2065 2070

Gly Leu Leu Tyr Phe Val Ala Gln Phe Ile Ser Thr Phe Gly Ser
2075 2080 2085

```
Phe Leu  Gly Phe His Gln Lys  Gln Trp Phe Leu His  Phe Val Pro
    2090                 2095                 2100

Phe Asp  Val Leu Cys Asn Glu  Phe Leu Ala Thr Phe  Ile Val Cys
    2105                 2110                 2115

Lys Ile  Val Leu Phe Val Arg  His Ile Ile Val Gly  Cys Asn Asn
    2120                 2125                 2130

Ala Asp  Cys Val Ala Cys Ser  Lys Ser Ala Arg Leu  Lys Arg Val
    2135                 2140                 2145

Pro Leu  Gln Thr Ile Ile Asn  Gly Met His Lys Ser  Phe Tyr Val
    2150                 2155                 2160

Asn Ala  Asn Gly Gly Thr Cys  Phe Cys Asn Lys His  Asn Phe Phe
    2165                 2170                 2175

Cys Val  Asn Cys Asp Ser Phe  Gly Pro Gly Asn Thr  Phe Ile Asn
    2180                 2185                 2190

Gly Asp  Ile Ala Arg Glu Leu  Gly Asn Val Val Lys  Thr Ala Val
    2195                 2200                 2205

Gln Pro  Thr Ala Pro Ala Tyr  Val Ile Ile Asp Lys  Val Asp Phe
    2210                 2215                 2220

Val Asn  Gly Phe Tyr Arg Leu  Tyr Ser Gly Asp Thr  Phe Trp Arg
    2225                 2230                 2235

Tyr Asp  Phe Asp Ile Thr Glu  Ser Lys Tyr Ser Cys  Lys Glu Val
    2240                 2245                 2250

Leu Lys  Asn Cys Asn Val Leu  Glu Asn Phe Ile Val  Tyr Asn Asn
    2255                 2260                 2265

Ser Gly  Ser Asn Ile Thr Gln  Ile Lys Asn Ala Cys  Val Tyr Phe
    2270                 2275                 2280

Ser Gln  Leu Leu Cys Glu Pro  Ile Lys Leu Val Asn  Ser Glu Leu
    2285                 2290                 2295
```

```
Leu Ser  Thr Leu Ser Val Asp  Phe Asn Gly Val Leu  His Lys Ala
    2300                 2305                 2310

Tyr Val  Asp Val Leu Cys Asn  Ser Phe Phe Lys Glu  Leu Thr Ala
    2315                 2320                 2325

Asn Met  Ser Met Ala Glu Cys  Lys Ala Thr Leu Gly  Leu Thr Val
    2330                 2335                 2340

Ser Asp  Asp Asp Phe Val Ser  Ala Val Ala Asn Ala  His Arg Tyr
    2345                 2350                 2355

Asp Val  Leu Leu Ser Asp Leu  Ser Phe Asn Asn Phe  Phe Ile Ser
    2360                 2365                 2370

Tyr Ala  Lys Pro Glu Asp Lys  Leu Ser Val Tyr Asp  Ile Ala Cys
    2375                 2380                 2385

Cys Met  Arg Ala Gly Ser Lys  Val Val Asn His Asn  Val Leu Ile
    2390                 2395                 2400

Lys Glu  Ser Ile Pro Ile Val  Trp Gly Val Lys Asp  Phe Asn Thr
    2405                 2410                 2415

Leu Ser  Gln Glu Gly Lys Lys  Tyr Leu Val Lys Thr  Thr Lys Ala
    2420                 2425                 2430

Lys Gly  Leu Thr Phe Leu Leu  Thr Phe Asn Asp Asn  Gln Ala Ile
    2435                 2440                 2445

Thr Gln  Val Pro Ala Thr Ser  Ile Val Ala Lys Gln  Gly Ala Gly
    2450                 2455                 2460

Phe Lys  Arg Thr Tyr Asn Phe  Leu Trp Tyr Val Cys  Leu Phe Val
    2465                 2470                 2475

Val Ala  Leu Phe Ile Gly Val  Ser Phe Ile Asp Tyr  Thr Thr Thr
    2480                 2485                 2490

Val Thr  Ser Phe His Gly Tyr  Asp Phe Lys Tyr Ile  Glu Asn Gly
    2495                 2500                 2505
```

```
Gln Leu  Lys Val Phe Glu Ala  Pro Leu His Cys Val  Arg Asn Val
    2510                2515                2520

Phe Asp  Asn Phe Asn Gln Trp  His Glu Ala Lys Phe  Gly Val Val
    2525                2530                2535

Thr Thr  Asn Ser Asp Lys Cys  Pro Ile Val Val Gly  Val Ser Glu
    2540                2545                2550

Arg Ile  Asn Val Val Pro Gly  Val Pro Thr Asn Val  Tyr Leu Val
    2555                2560                2565

Gly Lys  Thr Leu Val Phe Thr  Leu Gln Ala Ala Phe  Gly Asn Thr
    2570                2575                2580

Gly Val  Cys Tyr Asp Phe Asp  Gly Val Thr Thr Ser  Asp Lys Cys
    2585                2590                2595

Ile Phe  Asn Ser Ala Cys Thr  Arg Leu Glu Gly Leu  Gly Gly Asp
    2600                2605                2610

Asn Val  Tyr Cys Tyr Asn Thr  Asp Leu Ile Glu Gly  Ser Lys Pro
    2615                2620                2625

Tyr Ser  Thr Leu Gln Pro Asn  Ala Tyr Tyr Lys Tyr  Asp Ala Lys
    2630                2635                2640

Asn Tyr  Val Arg Phe Pro Glu  Ile Leu Ala Arg Gly  Phe Gly Leu
    2645                2650                2655

Arg Thr  Ile Arg Thr Leu Ala  Thr Arg Tyr Cys Arg  Val Gly Glu
    2660                2665                2670

Cys Arg  Asp Ser His Lys Gly  Val Cys Phe Gly Phe  Asp Lys Trp
    2675                2680                2685

Tyr Val  Asn Asp Gly Arg Val  Asp Asp Gly Tyr Ile  Cys Gly Asp
    2690                2695                2700

Gly Leu  Ile Asp Leu Leu Val  Asn Val Leu Ser Ile  Phe Ser Ser
    2705                2710                2715

Ser Phe  Ser Val Val Ala Met  Ser Gly His Met Leu  Phe Asn Phe
```

```
    2720                2725                2730

Leu Phe Ala Ala Phe Ile Thr Phe Leu Cys Phe Leu Val Thr Lys
    2735                2740                2745

Phe Lys Arg Val Phe Gly Asp Leu Ser Tyr Gly Val Phe Thr Val
    2750                2755                2760

Val Cys Ala Thr Leu Ile Asn Asn Ile Ser Tyr Val Val Thr Gln
    2765                2770                2775

Asn Leu Phe Phe Met Leu Leu Tyr Ala Ile Leu Tyr Phe Val Phe
    2780                2785                2790

Thr Arg Thr Val Arg Tyr Ala Trp Ile Trp His Ile Ala Tyr Ile
    2795                2800                2805

Val Ala Tyr Phe Leu Leu Ile Pro Trp Trp Leu Leu Thr Trp Phe
    2810                2815                2820

Ser Phe Ala Ala Phe Leu Glu Leu Leu Pro Asn Val Phe Lys Leu
    2825                2830                2835

Lys Ile Ser Thr Gln Leu Phe Glu Gly Asp Lys Phe Ile Gly Thr
    2840                2845                2850

Phe Glu Ser Ala Ala Ala Gly Thr Phe Val Leu Asp Met Arg Ser
    2855                2860                2865

Tyr Glu Arg Leu Ile Asn Thr Ile Ser Pro Glu Lys Leu Lys Asn
    2870                2875                2880

Tyr Ala Ala Ser Tyr Asn Lys Tyr Lys Tyr Tyr Ser Gly Ser Ala
    2885                2890                2895

Ser Glu Ala Asp Tyr Arg Cys Ala Cys Tyr Ala His Leu Ala Lys
    2900                2905                2910

Ala Met Leu Asp Tyr Ala Lys Asp His Asn Asp Met Leu Tyr Ser
    2915                2920                2925

Pro Pro Thr Ile Ser Tyr Asn Ser Thr Leu Gln Ser Gly Leu Lys
    2930                2935                2940
```

```
Lys Met  Ala Gln Pro Ser Gly  Cys Val Glu Arg Cys  Val Val Arg
    2945                 2950                 2955

Val Cys  Tyr Gly Ser Thr Val  Leu Asn Gly Val Trp  Leu Gly Asp
    2960                 2965                 2970

Thr Val  Thr Cys Pro Arg His  Val Ile Ala Pro Ser  Thr Thr Val
    2975                 2980                 2985

Leu Ile  Asp Tyr Asp His Ala  Tyr Ser Thr Met Arg  Leu His Asn
    2990                 2995                 3000

Phe Ser  Val Ser His Asn Gly  Val Phe Leu Gly Val  Val Gly Val
    3005                 3010                 3015

Thr Met  His Gly Ser Val Leu  Arg Ile Lys Val Ser  Gln Ser Asn
    3020                 3025                 3030

Val His  Thr Pro Lys His Val  Phe Lys Thr Leu Lys  Pro Gly Asp
    3035                 3040                 3045

Ser Phe  Asn Ile Leu Ala Cys  Tyr Glu Gly Ile Ala  Ser Gly Val
    3050                 3055                 3060

Phe Gly  Val Asn Leu Arg Thr  Asn Phe Thr Ile Lys  Gly Ser Phe
    3065                 3070                 3075

Ile Asn  Gly Ala Cys Gly Ser  Pro Gly Tyr Asn Val  Arg Asn Asp
    3080                 3085                 3090

Gly Thr  Val Glu Phe Cys Tyr  Leu His Gln Ile Glu  Leu Gly Ser
    3095                 3100                 3105

Gly Ala  His Val Gly Ser Asp  Phe Thr Gly Ser Val  Tyr Gly Asn
    3110                 3115                 3120

Phe Asp  Asp Gln Pro Ser Leu  Gln Val Glu Ser Ala  Asn Leu Met
    3125                 3130                 3135

Leu Ser  Asp Asn Val Val Ala  Phe Leu Tyr Ala Ala  Leu Leu Asn
    3140                 3145                 3150
```

```
Gly Cys  Arg Trp Trp Leu Cys  Ser Thr Arg Val Asn  Val Asp Gly
    3155                 3160                 3165

Phe Asn  Glu Trp Ala Met Ala  Asn Gly Tyr Thr Ser  Val Ser Ser
    3170                 3175                 3180

Val Glu  Cys Tyr Ser Ile Leu  Ala Ala Lys Thr Gly  Val Ser Val
    3185                 3190                 3195

Glu Gln  Leu Leu Ala Ser Ile  Gln His Leu His Glu  Gly Phe Gly
    3200                 3205                 3210

Gly Lys  Asn Ile Leu Gly Tyr  Ser Ser Leu Cys Asp  Glu Phe Thr
    3215                 3220                 3225

Leu Ala  Glu Val Val Lys Gln  Met Tyr Gly Val Asn  Leu Gln Ser
    3230                 3235                 3240

Gly Lys  Val Ile Phe Gly Leu  Lys Thr Met Phe Leu  Phe Ser Val
    3245                 3250                 3255

Phe Phe  Thr Met Phe Trp Ala  Glu Leu Phe Ile Tyr  Thr Asn Thr
    3260                 3265                 3270

Ile Trp  Ile Asn Pro Val Ile  Leu Thr Pro Ile Phe  Cys Leu Leu
    3275                 3280                 3285

Leu Phe  Leu Ser Leu Val Leu  Thr Met Phe Leu Lys  His Lys Phe
    3290                 3295                 3300

Leu Phe  Leu Gln Val Phe Leu  Leu Pro Thr Val Ile  Ala Thr Ala
    3305                 3310                 3315

Leu Tyr  Asn Cys Val Leu Asp  Tyr Tyr Ile Val Lys  Phe Leu Ala
    3320                 3325                 3330

Asp His  Phe Asn Tyr Asn Val  Ser Val Leu Gln Met  Asp Val Gln
    3335                 3340                 3345

Gly Leu  Val Asn Val Leu Val  Cys Leu Phe Val Val  Phe Leu His
    3350                 3355                 3360
```

```
Thr Trp  Arg Phe Ser Lys Glu  Arg Phe Thr His Trp  Phe Thr Tyr
    3365                 3370                 3375

Val Cys  Ser Leu Ile Ala Val  Ala Tyr Thr Tyr Phe  Tyr Ser Gly
    3380                 3385                 3390

Asp Phe  Leu Ser Leu Leu Val  Met Phe Leu Cys Ala  Ile Ser Ser
    3395                 3400                 3405

Asp Trp  Tyr Ile Gly Ala Ile  Val Phe Arg Leu Ser  Arg Leu Ile
    3410                 3415                 3420

Val Phe  Phe Ser Pro Glu Ser  Val Phe Ser Val Phe  Gly Asp Val
    3425                 3430                 3435

Lys Leu  Thr Leu Val Val Tyr  Leu Ile Cys Gly Tyr  Leu Val Cys
    3440                 3445                 3450

Thr Tyr  Trp Gly Ile Leu Tyr  Trp Phe Asn Arg Phe  Phe Lys Cys
    3455                 3460                 3465

Thr Met  Gly Val Tyr Asp Phe  Lys Val Ser Ala Ala  Glu Phe Lys
    3470                 3475                 3480

Tyr Met  Val Ala Asn Gly Leu  His Ala Pro His Gly  Pro Phe Asp
    3485                 3490                 3495

Ala Leu  Trp Leu Ser Phe Lys  Leu Leu Gly Ile Gly  Gly Asp Arg
    3500                 3505                 3510

Cys Ile  Lys Ile Ser Thr Val  Gln Ser Lys Leu Thr  Asp Leu Lys
    3515                 3520                 3525

Cys Thr  Asn Val Val Leu Leu  Gly Cys Leu Ser Ser  Met Asn Ile
    3530                 3535                 3540

Ala Ala  Asn Ser Ser Glu Trp  Ala Tyr Cys Val Asp  Leu His Asn
    3545                 3550                 3555

Lys Ile  Asn Leu Cys Asp Asp  Pro Glu Lys Ala Gln  Ser Met Leu
    3560                 3565                 3570

Leu Ala  Leu Leu Ala Phe Phe  Leu Ser Lys His Ser  Asp Phe Gly
```

3575 3580 3585

Leu Asp Gly Leu Ile Asp Ser Tyr Phe Asp Asn Ser Ser Thr Leu
3590 3595 3600

Gln Ser Val Ala Ser Ser Phe Val Ser Met Pro Ser Tyr Ile Ala
3605 3610 3615

Tyr Glu Asn Ala Arg Gln Ala Tyr Glu Asp Ala Ile Ala Asn Gly
3620 3625 3630

Ser Ser Ser Gln Leu Ile Lys Gln Leu Lys Arg Ala Met Asn Ile
3635 3640 3645

Ala Lys Ser Glu Phe Asp His Glu Ile Ser Val Gln Lys Lys Ile
3650 3655 3660

Asn Arg Met Ala Glu Gln Ala Ala Thr Gln Met Tyr Lys Glu Ala
3665 3670 3675

Arg Ser Val Asn Arg Lys Ser Lys Val Ile Ser Ala Met His Ser
3680 3685 3690

Leu Leu Phe Gly Met Leu Arg Arg Leu Asp Met Ser Ser Val Glu
3695 3700 3705

Thr Val Leu Asn Leu Ala Arg Asp Gly Val Val Pro Leu Ser Val
3710 3715 3720

Ile Pro Ala Thr Ser Ala Ser Lys Leu Thr Ile Val Ser Pro Asp
3725 3730 3735

Leu Glu Ser Tyr Ser Lys Ile Val Cys Asp Gly Ser Val His Tyr
3740 3745 3750

Ala Gly Val Val Trp Thr Leu Asn Asp Val Lys Asp Asn Asp Gly
3755 3760 3765

Arg Pro Val His Val Lys Glu Ile Thr Lys Glu Asn Val Glu Thr
3770 3775 3780

Leu Thr Trp Pro Leu Ile Leu Asn Cys Glu Arg Val Val Lys Leu
3785 3790 3795

```
Gln Asn  Asn Glu Ile Met Pro  Gly Lys Leu Lys Gln  Lys Pro Met
    3800                 3805                 3810

Lys Ala  Glu Gly Asp Gly Gly  Val Leu Gly Asp Gly  Asn Ala Leu
    3815                 3820                 3825

Tyr Asn  Thr Glu Gly Gly Lys  Thr Phe Met Tyr Ala  Tyr Ile Ser
    3830                 3835                 3840

Asn Lys  Ala Asp Leu Lys Phe  Val Lys Trp Glu Tyr  Glu Gly Gly
    3845                 3850                 3855

Cys Asn  Thr Ile Glu Leu Asp  Ser Pro Cys Arg Phe  Met Val Glu
    3860                 3865                 3870

Thr Pro  Asn Gly Pro Gln Val  Lys Tyr Leu Tyr Phe  Val Lys Asn
    3875                 3880                 3885

Leu Asn  Thr Leu Arg Arg Gly  Ala Val Leu Gly Phe  Ile Gly Ala
    3890                 3895                 3900

Thr Ile  Arg Leu Gln Ala Gly  Lys Gln Thr Glu Leu  Ala Val Asn
    3905                 3910                 3915

Ser Gly  Leu Leu Thr Ala Cys  Ala Phe Ser Val Asp  Pro Ala Thr
    3920                 3925                 3930

Thr Tyr  Leu Glu Ala Val Lys  His Gly Ala Lys Pro  Val Ser Asn
    3935                 3940                 3945

Cys Ile  Lys Met Leu Ser Asn  Gly Ala Gly Asn Gly  Gln Ala Ile
    3950                 3955                 3960

Thr Thr  Ser Val Asp Ala Asn  Thr Asn Gln Asp Ser  Tyr Gly Gly
    3965                 3970                 3975

Ala Ser  Ile Cys Leu Tyr Cys  Arg Ala His Val Pro  His Pro Ser
    3980                 3985                 3990

Met Asp  Gly Tyr Cys Lys Phe  Lys Gly Lys Cys Val  Gln Val Pro
    3995                 4000                 4005
```

Ile Gly Cys Leu Asp Pro Ile Arg Phe Cys Leu Glu Asn Asn Val
4010 4015 4020

Cys Asn Val Cys Gly Cys Trp Leu Gly His Gly Cys Ala Cys Asp
4025 4030 4035

Arg Thr Thr Ile Gln Ser Val Asp Ile Ser Tyr Leu Asn Glu Gln
4040 4045 4050

Gly Val Leu Val Gln Leu Asp
4055 4060

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Ser Gly Phe Gly Phe Ala Ile Pro Ser Val Ala Val Arg Thr Tyr Ser
20 25 30

Glu Ala Ala Ala Gln Gly Phe Gln Ala Cys Arg Phe Val Ala Phe Gly
35 40 45

Leu Gln Asp Cys Val Thr Gly Ile Asn Asp Asp Asp Tyr Val Ile Ala
50 55 60

Leu Thr Gly Thr Asn Gln Leu Cys Ala Lys Ile Leu Pro Phe Ser Asp
65 70 75 80

Arg Pro Leu Asn Leu Arg Gly Trp Leu Ile Phe Ser Asn Ser Asn Tyr
85 90 95

Val Leu Gln Asp Phe Asp Val Val Phe Gly His Gly Ala Gly Ser Val
100 105 110

```
Val Phe Val Asp Lys Tyr Met Cys Gly Phe Asp Gly Lys Pro Val Leu
        115                 120                 125

Pro Lys Asn Met Trp Glu Phe Arg Asp Tyr Phe Asn Asn Asn Thr Asp
    130                 135                 140

Ser Ile Val Ile Gly Gly Val Thr Tyr Gln Leu Ala Trp Asp Val Ile
145                 150                 155                 160

Arg Lys Asp Leu Ser Tyr Glu Gln Gln Asn Val Leu Ala Ile Glu Ser
                165                 170                 175

Ile His Tyr Leu Gly Thr Thr Gly His Thr Leu Lys Ser Gly Cys Lys
            180                 185                 190

Leu Thr Asn Ala Lys Pro Pro Lys Tyr Ser Ser Lys Val Val Leu Ser
        195                 200                 205

Gly Glu Trp Asn Ala Val Tyr Arg Ala Phe Gly Ser Pro Phe Ile Thr
    210                 215                 220

Asn Gly Met Ser Leu Leu Asp Ile Ile Val Lys Pro Val Phe Phe Asn
225                 230                 235                 240

Ala Phe Val Lys Cys Asn Cys Gly Ser Glu Ser Trp Ser Val Gly Ala
                245                 250                 255

Trp Asp Gly Tyr Leu Ser Ser Cys Cys Gly Thr Pro Ala Lys Lys Leu
            260                 265                 270

Cys Val Val Pro Gly Asn Val Val Pro Gly Asp Val Ile Ile Thr Ser
        275                 280                 285

Thr Ser Ala Gly Cys Gly Val Lys Tyr Tyr Ala Gly Leu Val Val Lys
    290                 295                 300

His Ile Thr Asn Ile Thr Gly Val Ser Leu Trp Arg Val Thr Ala Val
305                 310                 315                 320

His Ser Asp Gly Met Phe Val Ala Ser Ser Ser Tyr Asp Ala Leu Leu
                325                 330                 335
```

```
His Arg Asn Ser Leu Asp Pro Phe Cys Phe Asp Val Asn Thr Leu Leu
            340                 345                 350

Ser Asn Gln Leu Arg Leu Ala Phe Leu Gly Ala Ser Val Thr Glu Asp
        355                 360                 365

Val Lys Phe Ala Ala Ser Thr Gly Val Ile Asp Ile Ser Ala Gly Met
    370                 375                 380

Phe Gly Leu Tyr Asp Asp Ile Leu Thr Asn Asn Lys Pro Trp Phe Val
385                 390                 395                 400

Arg Lys Ala Ser Gly Leu Phe Asp Ala Ile Trp Asp Ala Phe Val Ala
                405                 410                 415

Ala Ile Lys Leu Val Pro Thr Thr Thr Gly Val Leu Val Arg Phe Val
            420                 425                 430

Lys Ser Ile Ala Ser Thr Val Leu Thr Val Ser Asn Gly Val Ile Ile
        435                 440                 445

Met Cys Ala Asp Val Pro Asp Ala Phe Gln Ser Val Tyr Arg Thr Phe
    450                 455                 460

Thr Gln Ala Ile Cys Ala Ala Phe Asp Phe Ser Leu Asp Val Phe Lys
465                 470                 475                 480

Ile Gly Asp Val Lys Phe Lys Arg Leu Gly Asp Tyr Val Leu Thr Glu
                485                 490                 495

Asn Ala Leu Val Arg Leu Thr Thr Glu Val Val Arg Gly Val Arg Asp
            500                 505                 510

Ala Arg Ile Lys Lys Ala Met Phe Thr Lys Val Val Val Gly Pro Thr
        515                 520                 525

Thr Glu Val Lys Phe Ser Val Ile Glu Leu Ala Thr Val Asn Leu Arg
    530                 535                 540

Leu Val Asp Cys Ala Pro Val Val Cys Pro Lys Gly Lys Ile Val Val
545                 550                 555                 560

Ile Ala Gly Gln Ala Phe Phe Tyr Ser Gly Gly Phe Tyr Arg Phe Met
```

```
                565                 570                 575

Val Asp Pro Thr Thr Val Leu Asn Asp Pro Val Phe Thr Gly Asp Leu
            580                 585                 590

Phe Tyr Thr Ile Lys Phe Ser Gly Phe Lys Leu Asp Gly Phe Asn His
        595                 600                 605

Gln Phe Val Thr Ala Ser Ser Ala Thr Asp Ala Ile Ile Ala Val Glu
    610                 615                 620

Leu Leu Leu Leu Asp Phe Lys Thr Ala Val Phe Val Tyr Thr Cys Val
625                 630                 635                 640

Val Asp Gly Cys Ser Val Ile Val Arg Arg Asp Ala Thr Phe Ala Thr
                645                 650                 655

His Val Cys Phe Lys Asp Cys Tyr Asn Val Trp Glu Gln Phe Cys Ile
            660                 665                 670

Asp Asn Cys Gly Glu Pro Trp Phe Leu Thr Asp Tyr Asn Ala Ile Leu
        675                 680                 685

Gln Ser Asn Asn Pro Gln Cys Ala Ile Val Gln Ala Ser Glu Ser Lys
    690                 695                 700

Val Leu Leu Glu Arg Phe Leu Pro Lys Cys Pro Glu Ile Leu Leu Ser
705                 710                 715                 720

Ile Asp Asp Gly His Leu Trp Asn Leu Phe Val Glu Lys Phe Asn Phe
                725                 730                 735

Val Thr Asp Trp Leu Lys Thr Leu Lys Leu Thr Leu Thr Ser Asn Gly
            740                 745                 750

Leu Leu Gly Asn Cys Ala Lys Arg Phe Arg Arg Val Leu Val Lys Leu
        755                 760                 765

Leu Asp Val Tyr Asn Gly Phe Leu Glu Thr Val Cys Ser Val Ala Tyr
    770                 775                 780

Thr Ala Gly Val Cys Ile Lys Tyr Tyr Ala Val Asn Val Pro Tyr Val
785                 790                 795                 800
```

```
Val Ile Ser Gly Phe Val Ser Arg Val Ile Arg Arg Glu Arg Cys Asp
                805                 810                 815

Met Thr Phe Pro Cys Val Ser Cys Val Thr Phe Phe Tyr Glu Phe Leu
            820                 825                 830

Asp Thr Cys Phe Gly Val Ser Lys Pro Asn Ala Ile Asp Val Glu His
        835                 840                 845

Leu Glu Leu Lys Glu Thr Val Phe Val Glu Pro Lys Asp Gly Gly Gln
    850                 855                 860

Phe Phe Val Ser Gly Asp Tyr Leu Trp Tyr Val Val Asp Asp Ile Tyr
865                 870                 875                 880

Tyr Pro Ala Ser Cys Asn Gly Val Leu Pro Val Ala Phe Thr Lys Leu
                885                 890                 895

Ala Gly Gly Lys Ile Ser Phe Ser Asp Asp Val Ile Val His Asp Val
            900                 905                 910

Glu Pro Thr His Lys Val Lys Leu Ile Phe Glu Phe Glu Asp Asp Val
        915                 920                 925

Val Thr Ser Leu Cys Lys Lys Ser Phe Gly Lys Ser Ile Ile Tyr Thr
    930                 935                 940

Gly Asp Trp Glu Gly Leu His Glu Val Leu Thr Ser Ala Met Asn Val
945                 950                 955                 960

Ile Gly Gln His Ile Lys Leu Pro Gln Phe Tyr Ile Tyr Asp Glu Glu
                965                 970                 975

Gly Gly Tyr Asp Val Ser Lys Pro Val Met Ile Ser Gln Trp Pro Ile
            980                 985                 990

Ser Asn Asp Ser Asn Gly Cys Val  Val Glu Ala Ser Thr  Asp Phe His
        995                 1000                1005

Gln Leu  Glu Cys Ile Val Asp  Asp Ser Val Arg Glu  Glu Val Asp
    1010                1015                1020
```

Ile Ile Glu Gln Pro Phe Glu Glu Val Glu His Val Leu Ser Ile
1025 1030 1035

Lys Gln Pro Phe Ser Phe Ser Phe Arg Asp Glu Leu Gly Val Arg
1040 1045 1050

Val Leu Asp Gln Ser Asp Asn Asn Cys Trp Ile Ser Thr Thr Leu
1055 1060 1065

Val Gln Leu Gln Leu Thr Lys Leu Leu Asp Asp Ser Ile Glu Met
1070 1075 1080

Gln Leu Phe Lys Val Gly Lys Val Asp Ser Ile Val Gln Lys Cys
1085 1090 1095

Tyr Glu Leu Ser His Leu Ile Ser Gly Ser Leu Gly Asp Ser Gly
1100 1105 1110

Lys Leu Leu Ser Glu Leu Leu Lys Glu Lys Tyr Thr Cys Ser Ile
1115 1120 1125

Thr Phe Glu Met Ser Cys Asp Cys Gly Lys Lys Phe Asp Asp Gln
1130 1135 1140

Val Gly Cys Leu Phe Trp Ile Met Pro Tyr Thr Lys Leu Phe Gln
1145 1150 1155

Lys Gly Glu Cys Cys Ile Cys His Lys Met Gln Thr Tyr Lys Leu
1160 1165 1170

Val Ser Met Lys Gly Thr Gly Val Phe Val Gln Asp Pro Ala Pro
1175 1180 1185

Ile Asp Ile Asp Ala Phe Pro Val Lys Pro Ile Cys Ser Ser Val
1190 1195 1200

Tyr Leu Gly Val Lys Gly Ser Gly His Tyr Gln Thr Asn Leu Tyr
1205 1210 1215

Ser Phe Asn Lys Ala Ile Asp Gly Phe Gly Val Phe Asp Ile Lys
1220 1225 1230

```
Asn Ser  Ser Val Asn Thr Val  Cys Phe Val Asp Val  Asp Phe His
    1235                 1240                 1245

Ser Val  Glu Ile Glu Ala Gly  Glu Val Lys Pro Phe  Ala Val Tyr
    1250                 1255                 1260

Lys Asn  Val Lys Phe Tyr Leu  Gly Asp Ile Ser His  Leu Val Asn
    1265                 1270                 1275

Cys Val  Ser Phe Asp Phe Val  Val Asn Ala Ala Asn  Glu Asn Leu
    1280                 1285                 1290

Leu His  Gly Gly Gly Val Ala  Arg Ala Ile Asp Ile  Leu Thr Glu
    1295                 1300                 1305

Gly Gln  Leu Gln Ser Leu Ser  Lys Asp Tyr Ile Ser  Ser Asn Gly
    1310                 1315                 1320

Pro Leu  Lys Val Gly Ala Gly  Val Met Leu Glu Cys  Glu Lys Phe
    1325                 1330                 1335

Asn Val  Phe Asn Val Val Gly  Pro Arg Thr Gly Lys  His Glu His
    1340                 1345                 1350

Ser Leu  Leu Val Glu Ala Tyr  Asn Ser Ile Leu Phe  Glu Asn Gly
    1355                 1360                 1365

Ile Pro  Leu Met Pro Leu Leu  Ser Cys Gly Ile Phe  Gly Val Arg
    1370                 1375                 1380

Ile Glu  Asn Ser Leu Lys Ala  Leu Phe Ser Cys Asp  Ile Asn Lys
    1385                 1390                 1395

Pro Leu  Gln Val Phe Val Tyr  Ser Ser Asn Glu Glu  Gln Ala Val
    1400                 1405                 1410

Leu Lys  Phe Leu Asp Gly Leu  Asp Leu Thr Pro Val  Ile Asp Asp
    1415                 1420                 1425

Val Asp  Val Val Lys Pro Phe  Arg Val Glu Gly Asn  Phe Ser Phe
    1430                 1435                 1440

Phe Asp  Cys Gly Val Asn Ala  Leu Asp Gly Asp Ile  Tyr Leu Leu
```

1445 1450 1455

Phe Thr Asn Ser Ile Leu Met Leu Asp Lys Gln Gly Gln Leu Leu
1460 1465 1470

Asp Thr Lys Leu Asn Gly Ile Leu Gln Gln Ala Ala Leu Asp Tyr
1475 1480 1485

Leu Ala Thr Val Lys Thr Val Pro Ala Gly Asn Leu Val Lys Leu
1490 1495 1500

Phe Val Glu Ser Cys Thr Ile Tyr Met Cys Val Val Pro Ser Ile
1505 1510 1515

Asn Asp Leu Ser Phe Asp Lys Asn Leu Gly Arg Cys Val Arg Lys
1520 1525 1530

Leu Asn Arg Leu Lys Thr Cys Val Ile Ala Asn Val Pro Ala Ile
1535 1540 1545

Asp Val Leu Lys Lys Leu Leu Ser Ser Leu Thr Leu Thr Val Lys
1550 1555 1560

Phe Val Val Glu Ser Asn Val Met Asp Val Asn Asp Cys Phe Lys
1565 1570 1575

Asn Asp Asn Val Val Leu Lys Ile Thr Glu Asp Gly Ile Asn Val
1580 1585 1590

Lys Asp Val Val Val Glu Ser Ser Lys Ser Leu Gly Lys Gln Leu
1595 1600 1605

Gly Val Val Ser Asp Gly Val Asp Ser Phe Glu Gly Val Leu Pro
1610 1615 1620

Ile Asn Thr Asp Thr Val Leu Ser Val Ala Pro Glu Val Asp Trp
1625 1630 1635

Val Ala Phe Tyr Gly Phe Glu Lys Ala Ala Leu Phe Ala Ser Leu
1640 1645 1650

Asp Val Lys Pro Tyr Gly Tyr Pro Asn Asp Phe Val Gly Gly Phe
1655 1660 1665

```
Arg Val  Leu Gly Thr Thr Asp  Asn Asn Cys Trp Val  Asn Ala Thr
    1670                 1675                 1680

Cys Ile  Ile Leu Gln Tyr Leu  Lys Pro Thr Phe Lys  Ser Lys Gly
    1685                 1690                 1695

Leu Asn  Val Leu Trp Asn Lys  Phe Val Thr Gly Asp  Val Gly Pro
    1700                 1705                 1710

Phe Val  Ser Phe Ile Tyr Phe  Ile Thr Met Ser Ser  Lys Gly Gln
    1715                 1720                 1725

Lys Gly  Asp Ala Glu Glu Ala  Leu Ser Lys Leu Ser  Glu Tyr Leu
    1730                 1735                 1740

Ile Ser  Asp Ser Ile Val Thr  Leu Glu Gln Tyr Ser  Thr Cys Asp
    1745                 1750                 1755

Ile Cys  Lys Ser Thr Val Val  Glu Val Lys Ser Ala  Ile Val Cys
    1760                 1765                 1770

Ala Ser  Val Leu Lys Asp Gly  Cys Asp Val Gly Phe  Cys Pro His
    1775                 1780                 1785

Arg His  Lys Leu Arg Ser Arg  Val Lys Phe Val Asn  Gly Arg Val
    1790                 1795                 1800

Val Ile  Thr Asn Val Gly Glu  Pro Ile Ile Ser Gln  Pro Ser Lys
    1805                 1810                 1815

Leu Leu  Asn Gly Ile Ala Tyr  Thr Thr Phe Ser Gly  Ser Phe Asp
    1820                 1825                 1830

Asn Gly  His Tyr Val Val Tyr  Asp Ala Ala Asn Asn  Ala Val Tyr
    1835                 1840                 1845

Asp Gly  Ala Arg Leu Phe Ser  Ser Asp Leu Ser Thr  Leu Ala Val
    1850                 1855                 1860

Thr Ala  Ile Val Val Val Gly  Gly Cys Val Thr Ser  Asn Val Pro
    1865                 1870                 1875
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```
Thr Ile  Val Ser Glu Lys Ile  Ser Val Met Asp Lys  Leu Asp Thr
    1880                 1885                 1890

Gly Ala  Gln Lys Phe Phe Gln  Phe Gly Asp Phe Val  Met Asn Asn
    1895                 1900                 1905

Ile Val  Leu Phe Leu Thr Trp  Leu Leu Ser Met Phe  Ser Leu Leu
    1910                 1915                 1920

Arg Thr  Ser Ile Met Lys His  Asp Ile Lys Val Ile  Ala Lys Ala
    1925                 1930                 1935

Pro Lys  Arg Thr Gly Val Ile  Leu Thr Arg Ser Phe  Lys Tyr Asn
    1940                 1945                 1950

Ile Arg  Ser Ala Leu Phe Val  Ile Lys Gln Lys Trp  Cys Val Ile
    1955                 1960                 1965

Val Thr  Leu Phe Lys Phe Leu  Leu Leu Leu Tyr Ala  Ile Tyr Ala
    1970                 1975                 1980

Leu Val  Phe Met Ile Val Gln  Phe Ser Pro Phe Asn  Ser Leu Leu
    1985                 1990                 1995

Cys Gly  Asp Ile Val Ser Gly  Tyr Glu Lys Ser Thr  Phe Asn Lys
    2000                 2005                 2010

Asp Ile  Tyr Cys Gly Asn Ser  Met Val Cys Lys Met  Cys Leu Phe
    2015                 2020                 2025

Ser Tyr  Gln Glu Phe Asn Asp  Leu Asp His Thr Ser  Leu Val Trp
    2030                 2035                 2040

Lys His  Ile Arg Asp Pro Ile  Leu Ile Ser Leu Gln  Pro Phe Val
    2045                 2050                 2055

Ile Leu  Val Ile Leu Leu Ile  Phe Gly Asn Met Tyr  Leu Arg Phe
    2060                 2065                 2070

Gly Leu  Leu Tyr Phe Val Ala  Gln Phe Ile Ser Thr  Phe Gly Ser
    2075                 2080                 2085
```

```
Phe Leu  Gly Phe His Gln Lys  Gln Trp Phe Leu His  Phe Val Pro
    2090                 2095                 2100

Phe Asp  Val Leu Cys Asn Glu  Phe Leu Ala Thr Phe  Ile Val Cys
    2105                 2110                 2115

Lys Ile  Val Leu Phe Val Arg  His Ile Ile Val Gly  Cys Asn Asn
    2120                 2125                 2130

Ala Asp  Cys Val Ala Cys Ser  Lys Ser Ala Arg Leu  Lys Arg Val
    2135                 2140                 2145

Pro Leu  Gln Thr Ile Ile Asn  Gly Met His Lys Ser  Phe Tyr Val
    2150                 2155                 2160

Asn Ala  Asn Gly Gly Thr Cys  Phe Cys Asn Lys His  Asn Phe Phe
    2165                 2170                 2175

Cys Val  Asn Cys Asp Ser Phe  Gly Pro Gly Asn Thr  Phe Ile Asn
    2180                 2185                 2190

Gly Asp  Ile Ala Arg Glu Leu  Gly Asn Val Val Lys  Thr Ala Val
    2195                 2200                 2205

Gln Pro  Thr Ala Pro Ala Tyr  Val Ile Ile Asp Lys  Val Asp Phe
    2210                 2215                 2220

Val Asn  Gly Phe Tyr Arg Leu  Tyr Ser Gly Asp Thr  Phe Trp Arg
    2225                 2230                 2235

Tyr Asp  Phe Asp Ile Thr Glu  Ser Lys Tyr Ser Cys  Lys Glu Val
    2240                 2245                 2250

Leu Lys  Asn Cys Asn Val Leu  Glu Asn Phe Ile Val  Tyr Asn Asn
    2255                 2260                 2265

Ser Gly  Ser Asn Ile Thr Gln  Ile Lys Asn Ala Cys  Val Tyr Phe
    2270                 2275                 2280

Ser Gln  Leu Leu Cys Glu Pro  Ile Lys Leu Val Asn  Ser Glu Leu
    2285                 2290                 2295

Leu Ser  Thr Leu Ser Val Asp  Phe Asn Gly Val Leu  His Lys Ala
```

2300 2305 2310

Tyr Val Asp Val Leu Cys Asn Ser Phe Phe Lys Glu Leu Thr Ala
2315 2320 2325

Asn Met Ser Met Ala Glu Cys Lys Ala Thr Leu Gly Leu Thr Val
2330 2335 2340

Ser Asp Asp Asp Phe Val Ser Ala Val Ala Asn Ala His Arg Tyr
2345 2350 2355

Asp Val Leu Leu Ser Asp Leu Ser Phe Asn Asn Phe Phe Ile Ser
2360 2365 2370

Tyr Ala Lys Pro Glu Asp Lys Leu Ser Val Tyr Asp Ile Ala Cys
2375 2380 2385

Cys Met Arg Ala Gly Ser Lys Val Val Asn His Asn Val Leu Ile
2390 2395 2400

Lys Glu Ser Ile Pro Ile Val Trp Gly Val Lys Asp Phe Asn Thr
2405 2410 2415

Leu Ser Gln Glu Gly Lys Lys Tyr Leu Val Lys Thr Thr Lys Ala
2420 2425 2430

Lys Gly Leu Thr Phe Leu Leu Thr Phe Asn Asp Asn Gln Ala Ile
2435 2440 2445

Thr Gln Val Pro Ala Thr Ser Ile Val Ala Lys Gln Gly Ala Gly
2450 2455 2460

Phe Lys Arg Thr Tyr Asn Phe Leu Trp Tyr Val Cys Leu Phe Val
2465 2470 2475

Val Ala Leu Phe Ile Gly Val Ser Phe Ile Asp Tyr Thr Thr Thr
2480 2485 2490

Val Thr Ser Phe His Gly Tyr Asp Phe Lys Tyr Ile Glu Asn Gly
2495 2500 2505

Gln Leu Lys Val Phe Glu Ala Pro Leu His Cys Val Arg Asn Val
2510 2515 2520

```
Phe Asp  Asn Phe Asn Gln Trp  His Glu Ala Lys Phe  Gly Val Val
    2525                 2530                 2535

Thr Thr  Asn Ser Asp Lys Cys  Pro Ile Val Val Gly  Val Ser Glu
    2540                 2545                 2550

Arg Ile  Asn Val Val Pro Gly  Val Pro Thr Asn Val  Tyr Leu Val
    2555                 2560                 2565

Gly Lys  Thr Leu Val Phe Thr  Leu Gln Ala Ala Phe  Gly Asn Thr
    2570                 2575                 2580

Gly Val  Cys Tyr Asp Phe Asp  Gly Val Thr Thr Ser  Asp Lys Cys
    2585                 2590                 2595

Ile Phe  Asn Ser Ala Cys Thr  Arg Leu Glu Gly Leu  Gly Gly Asp
    2600                 2605                 2610

Asn Val  Tyr Cys Tyr Asn Thr  Asp Leu Ile Glu Gly  Ser Lys Pro
    2615                 2620                 2625

Tyr Ser  Thr Leu Gln Pro Asn  Ala Tyr Tyr Lys Tyr  Asp Ala Lys
    2630                 2635                 2640

Asn Tyr  Val Arg Phe Pro Glu  Ile Leu Ala Arg Gly  Phe Gly Leu
    2645                 2650                 2655

Arg Thr  Ile Arg Thr Leu Ala  Thr Arg Tyr Cys Arg  Val Gly Glu
    2660                 2665                 2670

Cys Arg  Asp Ser His Lys Gly  Val Cys Phe Gly Phe  Asp Lys Trp
    2675                 2680                 2685

Tyr Val  Asn Asp Gly Arg Val  Asp Asp Gly Tyr Ile  Cys Gly Asp
    2690                 2695                 2700

Gly Leu  Ile Asp Leu Leu Val  Asn Val Leu Ser Ile  Phe Ser Ser
    2705                 2710                 2715

Ser Phe  Ser Val Val Ala Met  Ser Gly His Met Leu  Phe Asn Phe
    2720                 2725                 2730
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Leu Phe  Ala Ala Phe Ile Thr  Phe Leu Cys Phe Leu  Val Thr Lys
    2735                 2740                 2745

Phe Lys  Arg Val Phe Gly Asp  Leu Ser Tyr Gly Val  Phe Thr Val
    2750                 2755                 2760

Val Cys  Ala Thr Leu Ile Asn  Asn Ile Ser Tyr Val  Val Thr Gln
    2765                 2770                 2775

Asn Leu  Phe Phe Met Leu Leu  Tyr Ala Ile Leu Tyr  Phe Val Phe
    2780                 2785                 2790

Thr Arg  Thr Val Arg Tyr Ala  Trp Ile Trp His Ile  Ala Tyr Ile
    2795                 2800                 2805

Val Ala  Tyr Phe Leu Leu Ile  Pro Trp Trp Leu Leu  Thr Trp Phe
    2810                 2815                 2820

Ser Phe  Ala Ala Phe Leu Glu  Leu Leu Pro Asn Val  Phe Lys Leu
    2825                 2830                 2835

Lys Ile  Ser Thr Gln Leu Phe  Glu Gly Asp Lys Phe  Ile Gly Thr
    2840                 2845                 2850

Phe Glu  Ser Ala Ala Ala Gly  Thr Phe Val Leu Asp  Met Arg Ser
    2855                 2860                 2865

Tyr Glu  Arg Leu Ile Asn Thr  Ile Ser Pro Glu Lys  Leu Lys Asn
    2870                 2875                 2880

Tyr Ala  Ala Ser Tyr Asn Lys  Tyr Lys Tyr Tyr Ser  Gly Ser Ala
    2885                 2890                 2895

Ser Glu  Ala Asp Tyr Arg Cys  Ala Cys Tyr Ala His  Leu Ala Lys
    2900                 2905                 2910

Ala Met  Leu Asp Tyr Ala Lys  Asp His Asn Asp Met  Leu Tyr Ser
    2915                 2920                 2925

Pro Pro  Thr Ile Ser Tyr Asn  Ser Thr Leu Gln Ser  Gly Leu Lys
    2930                 2935                 2940
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```
Lys Met  Ala Gln Pro Ser Gly  Cys Val Glu Arg Cys  Val Val Arg
    2945                 2950                 2955

Val Cys  Tyr Gly Ser Thr Val  Leu Asn Gly Val Trp  Leu Gly Asp
    2960                 2965                 2970

Thr Val  Thr Cys Pro Arg His  Val Ile Ala Pro Ser  Thr Thr Val
    2975                 2980                 2985

Leu Ile  Asp Tyr Asp His Ala  Tyr Ser Thr Met Arg  Leu His Asn
    2990                 2995                 3000

Phe Ser  Val Ser His Asn Gly  Val Phe Leu Gly Val  Val Gly Val
    3005                 3010                 3015

Thr Met  His Gly Ser Val Leu  Arg Ile Lys Val Ser  Gln Ser Asn
    3020                 3025                 3030

Val His  Thr Pro Lys His Val  Phe Lys Thr Leu Lys  Pro Gly Asp
    3035                 3040                 3045

Ser Phe  Asn Ile Leu Ala Cys  Tyr Glu Gly Ile Ala  Ser Gly Val
    3050                 3055                 3060

Phe Gly  Val Asn Leu Arg Thr  Asn Phe Thr Ile Lys  Gly Ser Phe
    3065                 3070                 3075

Ile Asn  Gly Ala Cys Gly Ser  Pro Gly Tyr Asn Val  Arg Asn Asp
    3080                 3085                 3090

Gly Thr  Val Glu Phe Cys Tyr  Leu His Gln Ile Glu  Leu Gly Ser
    3095                 3100                 3105

Gly Ala  His Val Gly Ser Asp  Phe Thr Gly Ser Val  Tyr Gly Asn
    3110                 3115                 3120

Phe Asp  Asp Gln Pro Ser Leu  Gln Val Glu Ser Ala  Asn Leu Met
    3125                 3130                 3135

Leu Ser  Asp Asn Val Val Ala  Phe Leu Tyr Ala Ala  Leu Leu Asn
    3140                 3145                 3150

Gly Cys  Arg Trp Trp Leu Cys  Ser Thr Arg Val Asn  Val Asp Gly
```

3155 3160 3165

Phe Asn Glu Trp Ala Met Ala Asn Gly Tyr Thr Ser Val Ser Ser
3170 3175 3180

Val Glu Cys Tyr Ser Ile Leu Ala Ala Lys Thr Gly Val Ser Val
3185 3190 3195

Glu Gln Leu Leu Ala Ser Ile Gln His Leu His Glu Gly Phe Gly
3200 3205 3210

Gly Lys Asn Ile Leu Gly Tyr Ser Ser Leu Cys Asp Glu Phe Thr
3215 3220 3225

Leu Ala Glu Val Val Lys Gln Met Tyr Gly Val Asn Leu Gln Ser
3230 3235 3240

Gly Lys Val Ile Phe Gly Leu Lys Thr Met Phe Leu Phe Ser Val
3245 3250 3255

Phe Phe Thr Met Phe Trp Ala Glu Leu Phe Ile Tyr Thr Asn Thr
3260 3265 3270

Ile Trp Ile Asn Pro Val Ile Leu Thr Pro Ile Phe Cys Leu Leu
3275 3280 3285

Leu Phe Leu Ser Leu Val Leu Thr Met Phe Leu Lys His Lys Phe
3290 3295 3300

Leu Phe Leu Gln Val Phe Leu Leu Pro Thr Val Ile Ala Thr Ala
3305 3310 3315

Leu Tyr Asn Cys Val Leu Asp Tyr Tyr Ile Val Lys Phe Leu Ala
3320 3325 3330

Asp His Phe Asn Tyr Asn Val Ser Val Leu Gln Met Asp Val Gln
3335 3340 3345

Gly Leu Val Asn Val Leu Val Cys Leu Phe Val Val Phe Leu His
3350 3355 3360

Thr Trp Arg Phe Ser Lys Glu Arg Phe Thr His Trp Phe Thr Tyr
3365 3370 3375

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Val Cys  Ser Leu Ile Ala Val  Ala Tyr Thr Tyr Phe  Tyr Ser Gly
    3380                 3385                 3390

Asp Phe  Leu Ser Leu Leu Val  Met Phe Leu Cys Ala  Ile Ser Ser
    3395                 3400                 3405

Asp Trp  Tyr Ile Gly Ala Ile  Val Phe Arg Leu Ser  Arg Leu Ile
    3410                 3415                 3420

Val Phe  Phe Ser Pro Glu Ser  Val Phe Ser Val Phe  Gly Asp Val
    3425                 3430                 3435

Lys Leu  Thr Leu Val Val Tyr  Leu Ile Cys Gly Tyr  Leu Val Cys
    3440                 3445                 3450

Thr Tyr  Trp Gly Ile Leu Tyr  Trp Phe Asn Arg Phe  Phe Lys Cys
    3455                 3460                 3465

Thr Met  Gly Val Tyr Asp Phe  Lys Val Ser Ala Ala  Glu Phe Lys
    3470                 3475                 3480

Tyr Met  Val Ala Asn Gly Leu  His Ala Pro His Gly  Pro Phe Asp
    3485                 3490                 3495

Ala Leu  Trp Leu Ser Phe Lys  Leu Leu Gly Ile Gly  Gly Asp Arg
    3500                 3505                 3510

Cys Ile  Lys Ile Ser Thr Val  Gln Ser Lys Leu Thr  Asp Leu Lys
    3515                 3520                 3525

Cys Thr  Asn Val Val Leu Leu  Gly Cys Leu Ser Ser  Met Asn Ile
    3530                 3535                 3540

Ala Ala  Asn Ser Ser Glu Trp  Ala Tyr Cys Val Asp  Leu His Asn
    3545                 3550                 3555

Lys Ile  Asn Leu Cys Asp Asp  Pro Glu Lys Ala Gln  Ser Met Leu
    3560                 3565                 3570

Leu Ala  Leu Leu Ala Phe Phe  Leu Ser Lys His Ser  Asp Phe Gly
    3575                 3580                 3585
```

```
Leu Asp  Gly Leu Ile Asp Ser  Tyr Phe Asp Asn Ser  Ser Thr Leu
    3590                 3595                 3600

Gln Ser  Val Ala Ser Ser Phe  Val Ser Met Pro Ser  Tyr Ile Ala
    3605                 3610                 3615

Tyr Glu  Asn Ala Arg Gln Ala  Tyr Glu Asp Ala Ile  Ala Asn Gly
    3620                 3625                 3630

Ser Ser  Ser Gln Leu Ile Lys  Gln Leu Lys Arg Ala  Met Asn Ile
    3635                 3640                 3645

Ala Lys  Ser Glu Phe Asp His  Glu Ile Ser Val Gln  Lys Lys Ile
    3650                 3655                 3660

Asn Arg  Met Ala Glu Gln Ala  Ala Thr Gln Met Tyr  Lys Glu Ala
    3665                 3670                 3675

Arg Ser  Val Asn Arg Lys Ser  Lys Val Ile Ser Ala  Met His Ser
    3680                 3685                 3690

Leu Leu  Phe Gly Met Leu Arg  Arg Leu Asp Met Ser  Ser Val Glu
    3695                 3700                 3705

Thr Val  Leu Asn Leu Ala Arg  Asp Gly Val Val Pro  Leu Ser Val
    3710                 3715                 3720

Ile Pro  Ala Thr Ser Ala Ser  Lys Leu Thr Ile Val  Ser Pro Asp
    3725                 3730                 3735

Leu Glu  Ser Tyr Ser Lys Ile  Val Cys Asp Gly Ser  Val His Tyr
    3740                 3745                 3750

Ala Gly  Val Val Trp Thr Leu  Asn Asp Val Lys Asp  Asn Asp Gly
    3755                 3760                 3765

Arg Pro  Val His Val Lys Glu  Ile Thr Lys Glu Asn  Val Glu Thr
    3770                 3775                 3780

Leu Thr  Trp Pro Leu Ile Leu  Asn Cys Glu Arg Val  Val Lys Leu
    3785                 3790                 3795
```

```
Gln Asn  Asn Glu Ile Met Pro  Gly Lys Leu Lys Gln  Lys Pro Met
    3800                3805                3810

Lys Ala  Glu Gly Asp Gly Gly  Val Leu Gly Asp Gly  Asn Ala Leu
    3815                3820                3825

Tyr Asn  Thr Glu Gly Gly Lys  Thr Phe Met Tyr Ala  Tyr Ile Ser
    3830                3835                3840

Asn Lys  Ala Asp Leu Lys Phe  Val Lys Trp Glu Tyr  Glu Gly Gly
    3845                3850                3855

Cys Asn  Thr Ile Glu Leu Asp  Ser Pro Cys Arg Phe  Met Val Glu
    3860                3865                3870

Thr Pro  Asn Gly Pro Gln Val  Lys Tyr Leu Tyr Phe  Val Lys Asn
    3875                3880                3885

Leu Asn  Thr Leu Arg Arg Gly  Ala Val Leu Gly Phe  Ile Gly Ala
    3890                3895                3900

Thr Ile  Arg Leu Gln Ala Gly  Lys Gln Thr Glu Leu  Ala Val Asn
    3905                3910                3915

Ser Gly  Leu Leu Thr Ala Cys  Ala Phe Ser Val Asp  Pro Ala Thr
    3920                3925                3930

Thr Tyr  Leu Glu Ala Val Lys  His Gly Ala Lys Pro  Val Ser Asn
    3935                3940                3945

Cys Ile  Lys Met Leu Ser Asn  Gly Ala Gly Asn Gly  Gln Ala Ile
    3950                3955                3960

Thr Thr  Ser Val Asp Ala Asn  Thr Asn Gln Asp Ser  Tyr Gly Gly
    3965                3970                3975

Ala Ser  Ile Cys Leu Tyr Cys  Arg Ala His Val Pro  His Pro Ser
    3980                3985                3990

Met Asp  Gly Tyr Cys Lys Phe  Lys Gly Lys Cys Val  Gln Val Pro
    3995                4000                4005

Ile Gly  Cys Leu Asp Pro Ile  Arg Phe Cys Leu Glu  Asn Asn Val
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4010                4015                4020

Cys Asn  Val Cys Gly Cys Trp  Leu Gly His Gly Cys  Ala Cys Asp
4025                4030                4035

Arg Thr  Thr Ile Gln Ser Val  Asp Ile Ser Tyr Leu  Asn Glu Gln
4040                4045                4050

Gly Val  Leu Val Gln Leu Asp  Arg Ala Arg Gly Ser  Ser Ala Ala
4055                4060                4065

Arg Leu  Glu Pro Cys Asn Gly  Thr Asp Ile Asp Lys  Cys Val Arg
4070                4075                4080

Ala Phe  Asp Ile Tyr Asn Lys  Asn Val Ser Phe Leu  Gly Lys Cys
4085                4090                4095

Leu Lys  Met Asn Cys Val Arg  Phe Lys Asn Ala Asp  Leu Lys Asp
4100                4105                4110

Gly Tyr  Phe Val Ile Lys Arg  Cys Thr Lys Ser Val  Met Glu His
4115                4120                4125

Glu Gln  Ser Met Tyr Asn Leu  Leu Asn Phe Ser Gly  Ala Leu Ala
4130                4135                4140

Glu His  Asp Phe Phe Thr Trp  Lys Asp Gly Arg Val  Ile Tyr Gly
4145                4150                4155

Asn Val  Ser Arg His Asn Leu  Thr Lys Tyr Thr Met  Met Asp Leu
4160                4165                4170

Val Tyr  Ala Met Arg Asn Phe  Asp Glu Gln Asn Cys  Asp Val Leu
4175                4180                4185

Lys Glu  Val Leu Val Leu Thr  Gly Cys Cys Asp Asn  Ser Tyr Phe
4190                4195                4200

Asp Ser  Lys Gly Trp Tyr Asp  Pro Val Glu Asn Glu  Asp Ile His
4205                4210                4215

Arg Val  Tyr Ala Ser Leu Gly  Lys Ile Val Ala Arg  Ala Met Leu
4220                4225                4230
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```
Lys Cys  Val Ala Leu Cys Asp  Ala Met Val Ala Lys  Gly Val Val
    4235                 4240                 4245

Gly Val  Leu Thr Leu Asp Asn  Gln Asp Leu Asn Gly  Asn Phe Tyr
    4250                 4255                 4260

Asp Phe  Gly Asp Phe Val Val  Ser Leu Pro Asn Met  Gly Val Pro
    4265                 4270                 4275

Cys Cys  Thr Ser Tyr Tyr Ser  Tyr Met Met Pro Ile  Met Gly Leu
    4280                 4285                 4290

Thr Asn  Cys Leu Ala Ser Glu  Cys Phe Val Lys Ser  Asp Ile Phe
    4295                 4300                 4305

Gly Ser  Asp Phe Lys Thr Phe  Asp Leu Leu Lys Tyr  Asp Phe Thr
    4310                 4315                 4320

Glu His  Lys Glu Asn Leu Phe  Asn Lys Tyr Phe Lys  His Trp Ser
    4325                 4330                 4335

Phe Asp  Tyr His Pro Asn Cys  Cys Asp Cys Tyr Asp  Asp Met Cys
    4340                 4345                 4350

Val Ile  His Cys Ala Asn Phe  Asn Thr Leu Phe Ala  Thr Thr Ile
    4355                 4360                 4365

Pro Gly  Thr Ala Phe Gly Pro  Leu Cys Arg Lys Val  Phe Ile Asp
    4370                 4375                 4380

Gly Val  Pro Leu Val Thr Thr  Ala Gly Tyr His Phe  Lys Gln Leu
    4385                 4390                 4395

Gly Leu  Val Trp Asn Lys Asp  Val Asn Thr His Ser  Val Arg Leu
    4400                 4405                 4410

Thr Ile  Thr Glu Leu Leu Gln  Phe Val Thr Asp Pro  Ser Leu Ile
    4415                 4420                 4425

Ile Ala  Ser Ser Pro Ala Leu  Val Asp Gln Arg Thr  Ile Cys Phe
    4430                 4435                 4440
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```
Ser Val  Ala Ala Leu Ser Thr  Gly Leu Thr Asn Gln  Val Val Lys
    4445                 4450                 4455

Pro Gly  His Phe Asn Glu Glu  Phe Tyr Asn Phe Leu  Arg Leu Arg
    4460                 4465                 4470

Gly Phe  Phe Asp Glu Gly Ser  Glu Leu Thr Leu Lys  His Phe Phe
    4475                 4480                 4485

Phe Ala  Gln Asn Gly Asp Ala  Ala Val Lys Asp Phe  Asp Phe Tyr
    4490                 4495                 4500

Arg Tyr  Asn Lys Pro Thr Ile  Leu Asp Ile Cys Gln  Ala Arg Val
    4505                 4510                 4515

Thr Tyr  Lys Ile Val Ser Arg  Tyr Phe Asp Ile Tyr  Glu Gly Gly
    4520                 4525                 4530

Cys Ile  Lys Ala Cys Glu Val  Val Val Thr Asn Leu  Asn Lys Ser
    4535                 4540                 4545

Ala Gly  Trp Pro Leu Asn Lys  Phe Gly Lys Ala Ser  Leu Tyr Tyr
    4550                 4555                 4560

Glu Ser  Ile Ser Tyr Glu Glu  Gln Asp Ala Leu Phe  Ala Leu Thr
    4565                 4570                 4575

Lys Arg  Asn Val Leu Pro Thr  Met Thr Gln Leu Asn  Leu Lys Tyr
    4580                 4585                 4590

Ala Ile  Ser Gly Lys Glu Arg  Ala Arg Thr Val Gly  Gly Val Ser
    4595                 4600                 4605

Leu Leu  Ser Thr Met Thr Thr  Arg Gln Tyr His Gln  Lys His Leu
    4610                 4615                 4620

Lys Ser  Ile Val Asn Thr Arg  Asn Ala Thr Val Val  Ile Gly Thr
    4625                 4630                 4635

Thr Lys  Phe Tyr Gly Gly Trp  Asn Asn Met Leu Arg  Thr Leu Ile
    4640                 4645                 4650
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Asp Gly  Val Glu Asn Pro Met  Leu Met Gly Trp Asp  Tyr Pro Lys
    4655                 4660                 4665

Cys Asp  Arg Ala Leu Pro Asn  Met Ile Arg Met Ile  Ser Ala Met
    4670                 4675                 4680

Val Leu  Gly Ser Lys His Val  Asn Cys Cys Thr Ala  Thr Asp Arg
    4685                 4690                 4695

Phe Tyr  Arg Leu Gly Asn Glu  Leu Ala Gln Val Leu  Thr Glu Val
    4700                 4705                 4710

Val Tyr  Ser Asn Gly Gly Phe  Tyr Phe Lys Pro Gly  Gly Thr Thr
    4715                 4720                 4725

Ser Gly  Asp Ala Ser Thr Ala  Tyr Ala Asn Ser Ile  Phe Asn Ile
    4730                 4735                 4740

Phe Gln  Ala Val Ser Ser Asn  Ile Asn Arg Leu Leu  Ser Val Pro
    4745                 4750                 4755

Ser Asp  Ser Cys Asn Asn Val  Asn Val Arg Asp Leu  Gln Arg Arg
    4760                 4765                 4770

Leu Tyr  Asp Asn Cys Tyr Arg  Leu Thr Ser Val Glu  Glu Ser Phe
    4775                 4780                 4785

Ile Glu  Asp Tyr Tyr Gly Tyr  Leu Arg Lys His Phe  Ser Met Met
    4790                 4795                 4800

Ile Leu  Ser Asp Asp Gly Val  Val Cys Tyr Asn Lys  Asp Tyr Ala
    4805                 4810                 4815

Glu Leu  Gly Tyr Ile Ala Asp  Ile Ser Ala Phe Lys  Ala Thr Leu
    4820                 4825                 4830

Tyr Tyr  Gln Asn Asn Val Phe  Met Ser Thr Ser Lys  Cys Trp Val
    4835                 4840                 4845

Glu Glu  Asp Leu Thr Lys Gly  Pro His Glu Phe Cys  Ser Gln His
    4850                 4855                 4860

Thr Met  Gln Ile Val Asp Lys  Asp Gly Thr Tyr Tyr  Leu Pro Tyr
```

4865 4870 4875

Pro Asp Pro Ser Arg Ile Leu Ser Ala Gly Val Phe Val Asp Asp
4880 4885 4890

Val Val Lys Thr Asp Ala Val Val Leu Leu Glu Arg Tyr Val Ser
4895 4900 4905

Leu Ala Ile Asp Ala Tyr Pro Leu Ser Lys His Pro Asn Ser Glu
4910 4915 4920

Tyr Arg Lys Val Phe Tyr Val Leu Leu Asp Trp Val Lys His Leu
4925 4930 4935

Asn Lys Asn Leu Asn Glu Gly Val Leu Glu Ser Phe Ser Val Thr
4940 4945 4950

Leu Leu Asp Asn Gln Glu Asp Lys Phe Trp Cys Glu Asp Phe Tyr
4955 4960 4965

Ala Ser Met Tyr Glu Asn Ser Thr Ile Leu Gln Ala Ala Gly Leu
4970 4975 4980

Cys Val Val Cys Gly Ser Gln Thr Val Leu Arg Cys Gly Asp Cys
4985 4990 4995

Leu Arg Lys Pro Met Leu Cys Thr Lys Cys Ala Tyr Asp His Val
5000 5005 5010

Phe Gly Thr Asp His Lys Phe Ile Leu Ala Ile Thr Pro Tyr Val
5015 5020 5025

Cys Asn Ala Ser Gly Cys Gly Val Ser Asp Val Lys Lys Leu Tyr
5030 5035 5040

Leu Gly Gly Leu Asn Tyr Tyr Cys Thr Asn His Lys Pro Gln Leu
5045 5050 5055

Ser Phe Pro Leu Cys Ser Ala Gly Asn Ile Phe Gly Leu Tyr Lys
5060 5065 5070

Asn Ser Ala Thr Gly Ser Leu Asp Val Glu Val Phe Asn Arg Leu
5075 5080 5085

```
Ala Thr  Ser Asp Trp Thr Asp  Val Arg Asp Tyr Lys  Leu Ala Asn
    5090                 5095                 5100

Asp Val  Lys Asp Thr Leu Arg  Leu Phe Ala Ala Glu  Thr Ile Lys
    5105                 5110                 5115

Ala Lys  Glu Glu Ser Val Lys  Ser Ser Tyr Ala Phe  Ala Thr Leu
    5120                 5125                 5130

Lys Glu  Val Val Gly Pro Lys  Glu Leu Leu Leu Ser  Trp Glu Ser
    5135                 5140                 5145

Gly Lys  Val Lys Pro Pro Leu  Asn Arg Asn Ser Val  Phe Thr Cys
    5150                 5155                 5160

Phe Gln  Ile Ser Lys Asp Ser  Lys Phe Gln Ile Gly  Glu Phe Ile
    5165                 5170                 5175

Phe Glu  Lys Val Glu Tyr Gly  Ser Asp Thr Val Thr  Tyr Lys Ser
    5180                 5185                 5190

Thr Val  Thr Thr Lys Leu Val  Pro Gly Met Ile Phe  Val Leu Thr
    5195                 5200                 5205

Ser His  Asn Val Gln Pro Leu  Arg Ala Pro Thr Ile  Ala Asn Gln
    5210                 5215                 5220

Glu Lys  Tyr Ser Ser Ile Tyr  Lys Leu His Pro Ala  Phe Asn Val
    5225                 5230                 5235

Ser Asp  Ala Tyr Ala Asn Leu  Val Pro Tyr Tyr Gln  Leu Ile Gly
    5240                 5245                 5250

Lys Gln  Lys Ile Thr Thr Ile  Gln Gly Pro Pro Gly  Ser Gly Lys
    5255                 5260                 5265

Ser His  Cys Ser Ile Gly Leu  Gly Leu Tyr Tyr Pro  Gly Ala Arg
    5270                 5275                 5280

Ile Val  Phe Val Ala Cys Ala  His Ala Ala Val Asp  Ser Leu Cys
    5285                 5290                 5295
```

```
Ala Lys  Ala Met Thr Val Tyr  Ser Ile Asp Lys Cys  Thr Arg Ile
    5300                 5305                 5310

Ile Pro  Ala Arg Ala Arg Val  Glu Cys Tyr Ser Gly  Phe Lys Pro
    5315                 5320                 5325

Asn Asn  Thr Ser Ala Gln Tyr  Ile Phe Ser Thr Val  Asn Ala Leu
    5330                 5335                 5340

Pro Glu  Cys Asn Ala Asp Ile  Val Val Val Asp Glu  Val Ser Met
    5345                 5350                 5355

Cys Thr  Asn Tyr Asp Leu Ser  Val Ile Asn Gln Arg  Leu Ser Tyr
    5360                 5365                 5370

Lys His  Ile Val Tyr Val Gly  Asp Pro Gln Gln Leu  Pro Ala Pro
    5375                 5380                 5385

Arg Val  Met Ile Thr Lys Gly  Val Met Glu Pro Val  Asp Tyr Asn
    5390                 5395                 5400

Val Val  Thr Gln Arg Met Cys  Ala Ile Gly Pro Asp  Val Phe Leu
    5405                 5410                 5415

His Lys  Cys Tyr Arg Cys Pro  Ala Glu Ile Val Ile  Gln Phe Leu
    5420                 5425                 5430

Asn Leu  Phe Met Arg Thr Ser  Leu Ser Leu Leu Asn  Leu Leu Val
    5435                 5440                 5445

Asn Ser  Val Leu Lys Ser Phe  Leu Arg Val Met Tyr  Lys Val Asp
    5450                 5455                 5460

Asn Gly  Ser Ser Ile Asn Arg  Lys Gln Leu Glu Ile  Val Lys Leu
    5465                 5470                 5475

Phe Leu  Val Lys Asn Pro Ser  Trp Ser Lys Ala Val  Phe Ile Ser
    5480                 5485                 5490

Pro Tyr  Asn Ser Gln Asn Tyr  Val Ala Ser Arg Phe  Leu Gly Leu
    5495                 5500                 5505
```

```
Gln Ile  Gln Thr Val Asp Ser  Ser Gln Gly Ser Glu  Tyr Asp Tyr
    5510                 5515                 5520

Val Ile  Tyr Ala Gln Thr Ser  Asp Thr Ala His Ala  Cys Asn Val
    5525                 5530                 5535

Asn Arg  Phe Asn Val Ala Ile  Thr Arg Ala Lys Lys  Gly Ile Phe
    5540                 5545                 5550

Cys Val  Met Cys Asp Lys Thr  Leu Phe Asp Ser Leu  Lys Phe Phe
    5555                 5560                 5565

Glu Ile  Lys His Ala Asp Leu  His Ser Ser Gln Val  Cys Gly Leu
    5570                 5575                 5580

Phe Lys  Asn Cys Thr Arg Thr  Pro Leu Asn Leu Pro  Pro Thr His
    5585                 5590                 5595

Ala His  Thr Phe Leu Ser Leu  Ser Asp Gln Phe Lys  Thr Thr Gly
    5600                 5605                 5610

Asp Leu  Ala Val Gln Ile Gly  Ser Asn Asn Val Cys  Thr Tyr Glu
    5615                 5620                 5625

His Val  Ile Ser Phe Met Gly  Phe Arg Phe Asp Ile  Ser Ile Pro
    5630                 5635                 5640

Gly Ser  His Ser Leu Phe Cys  Thr Arg Asp Phe Ala  Ile Arg Asn
    5645                 5650                 5655

Val Arg  Gly Trp Leu Gly Met  Asp Val Glu Ser Ala  His Val Cys
    5660                 5665                 5670

Gly Asp  Asn Ile Gly Thr Asn  Val Pro Leu Gln Val  Gly Phe Ser
    5675                 5680                 5685

Asn Gly  Val Asn Phe Val Val  Gln Thr Glu Gly Cys  Val Ser Thr
    5690                 5695                 5700

Asn Phe  Gly Asp Val Ile Lys  Pro Val Cys Ala Lys  Ser Pro Pro
    5705                 5710                 5715

Gly Glu  Gln Phe Arg His Leu  Ile Pro Leu Leu Arg  Lys Gly Gln
```

```
    5720                5725                5730

Pro Trp Leu Ile Val Arg Arg Arg Ile Val Gln Met Ile Ser Asp
    5735                5740                5745

Tyr Leu Ser Asn Leu Ser Asp Ile Leu Val Phe Val Leu Trp Ala
    5750                5755                5760

Gly Ser Leu Glu Leu Thr Thr Met Arg Tyr Phe Val Lys Ile Gly
    5765                5770                5775

Pro Ile Lys Tyr Cys Tyr Cys Gly Asn Phe Ala Thr Cys Tyr Asn
    5780                5785                5790

Ser Val Ser Asn Glu Tyr Cys Cys Phe Lys His Ala Leu Gly Cys
    5795                5800                5805

Asp Tyr Val Tyr Asn Pro Tyr Ala Phe Asp Ile Gln Gln Trp Gly
    5810                5815                5820

Tyr Val Gly Ser Leu Ser Gln Asn His His Thr Phe Cys Asn Ile
    5825                5830                5835

His Arg Asn Glu His Asp Ala Ser Gly Asp Ala Val Met Thr Arg
    5840                5845                5850

Cys Leu Ala Val His Asp Cys Phe Val Lys Asn Val Asp Trp Thr
    5855                5860                5865

Val Thr Tyr Pro Phe Ile Ala Asn Glu Lys Phe Ile Asn Gly Cys
    5870                5875                5880

Gly Arg Asn Val Gln Gly His Val Val Arg Ala Ala Leu Lys Leu
    5885                5890                5895

Tyr Lys Pro Ser Val Ile His Asp Ile Gly Asn Pro Lys Gly Val
    5900                5905                5910

Arg Cys Ala Val Thr Asp Ala Lys Trp Tyr Cys Tyr Asp Lys Gln
    5915                5920                5925

Pro Val Asn Ser Asn Val Lys Leu Leu Asp Tyr Asp Tyr Ala Thr
    5930                5935                5940
```

```
His Gly  Gln Leu Asp Gly Leu  Cys Leu Phe Trp Asn  Cys Asn Val
    5945                 5950                 5955

Asp Met  Tyr Pro Glu Phe Ser  Ile Val Cys Arg Phe  Asp Thr Arg
    5960                 5965                 5970

Thr Arg  Ser Val Phe Asn Leu  Glu Gly Val Asn Gly  Gly Ser Leu
    5975                 5980                 5985

Tyr Val  Asn Lys His Ala Phe  His Thr Pro Ala Tyr  Asp Lys Arg
    5990                 5995                 6000

Ala Phe  Val Lys Leu Lys Pro  Met Pro Phe Phe Tyr  Phe Asp Asp
    6005                 6010                 6015

Ser Asp  Cys Asp Val Val Gln  Glu Gln Val Asn Tyr  Val Pro Leu
    6020                 6025                 6030

Arg Ala  Ser Ser Cys Val Thr  Arg Cys Asn Ile Gly  Gly Ala Val
    6035                 6040                 6045

Cys Ser  Lys His Ala Asn Leu  Tyr Gln Lys Tyr Val  Glu Ala Tyr
    6050                 6055                 6060

Asn Thr  Phe Thr Gln Ala Gly  Phe Asn Ile Trp Val  Pro His Ser
    6065                 6070                 6075

Phe Asp  Val Tyr Asn Leu Trp  Gln Ile Phe Ile Glu  Thr Asn Leu
    6080                 6085                 6090

Gln Ser  Leu Glu Asn Ile Ala  Phe Asn Val Val Lys  Lys Gly Cys
    6095                 6100                 6105

Phe Thr  Gly Val Asp Gly Glu  Leu Pro Val Ala Val  Val Asn Asp
    6110                 6115                 6120

Lys Val  Phe Val Arg Tyr Gly  Asp Val Asp Asn Leu  Val Phe Thr
    6125                 6130                 6135

Asn Lys  Thr Thr Leu Pro Thr  Asn Val Ala Phe Glu  Leu Phe Ala
    6140                 6145                 6150
```

Lys Arg Lys Met Gly Leu Thr Pro Pro Leu Ser Ile Leu Lys Asn
6155 6160 6165

Leu Gly Val Val Ala Thr Tyr Lys Phe Val Leu Trp Asp Tyr Glu
6170 6175 6180

Ala Glu Arg Pro Phe Thr Ser Tyr Thr Lys Ser Val Cys Lys Tyr
6185 6190 6195

Thr Asp Phe Asn Glu Asp Val Cys Val Cys Phe Asp Asn Ser Ile
6200 6205 6210

Gln Gly Ser Tyr Glu Arg Phe Thr Leu Thr Thr Asn Ala Val Leu
6215 6220 6225

Phe Ser Thr Val Val Ile Lys Asn Leu Thr Pro Ile Lys Leu Asn
6230 6235 6240

Phe Gly Met Leu Asn Gly Met Pro Val Ser Ser Ile Lys Gly Asp
6245 6250 6255

Lys Gly Val Glu Lys Leu Val Asn Trp Tyr Ile Tyr Val Arg Lys
6260 6265 6270

Asn Gly Gln Phe Gln Asp His Tyr Asp Gly Phe Tyr Thr Gln Gly
6275 6280 6285

Arg Asn Leu Ser Asp Phe Thr Pro Arg Ser Asp Met Glu Tyr Asp
6290 6295 6300

Phe Leu Asn Met Asp Met Gly Val Phe Ile Asn Lys Tyr Gly Leu
6305 6310 6315

Glu Asp Phe Asn Phe Glu His Val Val Tyr Gly Asp Val Ser Lys
6320 6325 6330

Thr Thr Leu Gly Gly Leu His Leu Leu Ile Ser Gln Phe Arg Leu
6335 6340 6345

Ser Lys Met Gly Val Leu Lys Ala Asp Asp Phe Val Thr Ala Ser
6350 6355 6360

```
Asp Thr  Thr Leu Arg Cys Cys  Thr Val Thr Tyr Leu  Asn Glu Leu
    6365                 6370                 6375

Ser Ser  Lys Val Val Cys Thr  Tyr Met Asp Leu Leu  Leu Asp Asp
    6380                 6385                 6390

Phe Val  Thr Ile Leu Lys Ser  Leu Asp Leu Gly Val  Ile Ser Lys
    6395                 6400                 6405

Val His  Glu Val Ile Ile Asp  Asn Lys Pro Tyr Arg  Trp Met Leu
    6410                 6415                 6420

Trp Cys  Lys Asp Asn His Leu  Ser Thr Phe Tyr Pro  Gln Leu Gln
    6425                 6430                 6435

Ser Ala  Glu Trp Lys Cys Gly  Tyr Ala Met Pro Gln  Ile Tyr Lys
    6440                 6445                 6450

Leu Gln  Arg Met Cys Leu Glu  Pro Cys Asn Leu Tyr  Asn Tyr Gly
    6455                 6460                 6465

Ala Gly  Ile Lys Leu Pro Ser  Gly Ile Met Leu Asn  Val Val Lys
    6470                 6475                 6480

Tyr Thr  Gln Leu Cys Gln Tyr  Leu Asn Ser Thr Thr  Met Cys Val
    6485                 6490                 6495

Pro His  Asn Met Arg Val Leu  His Tyr Gly Ala Gly  Ser Asp Lys
    6500                 6505                 6510

Gly Val  Ala Pro Gly Thr Thr  Val Leu Lys Arg Trp  Leu Pro Pro
    6515                 6520                 6525

Asp Ala  Ile Ile Ile Asp Asn  Asp Ile Asn Asp Tyr  Val Ser Asp
    6530                 6535                 6540

Ala Asp  Phe Ser Ile Thr Gly  Asp Cys Ala Thr Val  Tyr Leu Glu
    6545                 6550                 6555

Asp Lys  Phe Asp Leu Leu Ile  Ser Asp Met Tyr Asp  Gly Arg Ile
    6560                 6565                 6570

Lys Phe  Cys Asp Gly Glu Asn  Val Ser Lys Asp Gly  Phe Phe Thr
```

6575 6580 6585

Tyr Leu Asn Gly Val Ile Arg Glu Lys Leu Ala Ile Gly Gly Ser
6590 6595 6600

Val Ala Ile Lys Ile Thr Glu Tyr Ser Trp Asn Lys Tyr Leu Tyr
6605 6610 6615

Glu Leu Ile Gln Arg Phe Ala Phe Trp Thr Leu Phe Cys Thr Ser
6620 6625 6630

Val Asn Thr Ser Ser Ser Glu Ala Phe Leu Ile Gly Ile Asn Tyr
6635 6640 6645

Leu Gly Asp Phe Ile Gln Gly Pro Phe Ile Ala Gly Asn Thr Val
6650 6655 6660

His Ala Asn Tyr Ile Phe Trp Arg Asn Ser Thr Ile Met Ser Leu
6665 6670 6675

Ser Tyr Asn Ser Val Leu Asp Leu Ser Lys Phe Glu Cys Lys His
6680 6685 6690

Lys Ala Thr Val Val Val Thr Leu Lys Asp Ser Asp Val Asn Asp
6695 6700 6705

Met Val Leu Ser Leu Ile Lys Ser Gly Arg Leu Leu Leu Arg Asn
6710 6715 6720

Asn Gly Arg Phe Gly Gly Phe Ser Asn His Leu Val Ser Thr Lys
6725 6730 6735

<210> 58
<211> 2250
<212> PRT
<213> Human coronavirus

<220>
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<222> (1)..(2250)
<223> Adenosine diphosphate-ribose 1'-phosphatase

<400> 58

Ser Asn Asn Pro Gln Cys Ala Ile Val Gln Ala Ser Glu Ser Lys Val

```
1               5                  10                  15

Leu Leu Glu Arg Phe Leu Pro Lys Cys Pro Glu Ile Leu Leu Ser Ile
            20                  25                  30

Asp Asp Gly His Leu Trp Asn Leu Phe Val Glu Lys Phe Asn Phe Val
        35                  40                  45

Thr Asp Trp Leu Lys Thr Leu Lys Leu Thr Leu Thr Ser Asn Gly Leu
    50                  55                  60

Leu Gly Asn Cys Ala Lys Arg Phe Arg Arg Val Leu Val Lys Leu Leu
65                  70                  75                  80

Asp Val Tyr Asn Gly Phe Leu Glu Thr Val Cys Ser Val Ala Tyr Thr
                85                  90                  95

Ala Gly Val Cys Ile Lys Tyr Tyr Ala Val Asn Val Pro Tyr Val Val
            100                 105                 110

Ile Ser Gly Phe Val Ser Arg Val Ile Arg Arg Glu Arg Cys Asp Met
        115                 120                 125

Thr Phe Pro Cys Val Ser Cys Val Thr Phe Phe Tyr Glu Phe Leu Asp
    130                 135                 140

Thr Cys Phe Gly Val Ser Lys Pro Asn Ala Ile Asp Val Glu His Leu
145                 150                 155                 160

Glu Leu Lys Glu Thr Val Phe Val Glu Pro Lys Asp Gly Gly Gln Phe
                165                 170                 175

Phe Val Ser Gly Asp Tyr Leu Trp Tyr Val Val Asp Asp Ile Tyr Tyr
            180                 185                 190

Pro Ala Ser Cys Asn Gly Val Leu Pro Val Ala Phe Thr Lys Leu Ala
        195                 200                 205

Gly Gly Lys Ile Ser Phe Ser Asp Asp Val Ile Val His Asp Val Glu
    210                 215                 220

Pro Thr His Lys Val Lys Leu Ile Phe Glu Phe Glu Asp Asp Val Val
225                 230                 235                 240
```

```
Thr Ser Leu Cys Lys Lys Ser Phe Gly Lys Ser Ile Ile Tyr Thr Gly
                245                 250                 255

Asp Trp Glu Gly Leu His Glu Val Leu Thr Ser Ala Met Asn Val Ile
            260                 265                 270

Gly Gln His Ile Lys Leu Pro Gln Phe Tyr Ile Tyr Asp Glu Glu Gly
        275                 280                 285

Gly Tyr Asp Val Ser Lys Pro Val Met Ile Ser Gln Trp Pro Ile Ser
    290                 295                 300

Asn Asp Ser Asn Gly Cys Val Val Glu Ala Ser Thr Asp Phe His Gln
305                 310                 315                 320

Leu Glu Cys Ile Val Asp Asp Ser Val Arg Glu Glu Val Asp Ile Ile
                325                 330                 335

Glu Gln Pro Phe Glu Glu Val Glu His Val Leu Ser Ile Lys Gln Pro
            340                 345                 350

Phe Ser Phe Ser Phe Arg Asp Glu Leu Gly Val Arg Val Leu Asp Gln
        355                 360                 365

Ser Asp Asn Asn Cys Trp Ile Ser Thr Thr Leu Val Gln Leu Gln Leu
    370                 375                 380

Thr Lys Leu Leu Asp Asp Ser Ile Glu Met Gln Leu Phe Lys Val Gly
385                 390                 395                 400

Lys Val Asp Ser Ile Val Gln Lys Cys Tyr Glu Leu Ser His Leu Ile
                405                 410                 415

Ser Gly Ser Leu Gly Asp Ser Gly Lys Leu Leu Ser Glu Leu Leu Lys
            420                 425                 430

Glu Lys Tyr Thr Cys Ser Ile Thr Phe Glu Met Ser Cys Asp Cys Gly
        435                 440                 445

Lys Lys Phe Asp Asp Gln Val Gly Cys Leu Phe Trp Ile Met Pro Tyr
    450                 455                 460
```

```
Thr Lys Leu Phe Gln Lys Gly Glu Cys Cys Ile Cys His Lys Met Gln
465                 470                 475                 480

Thr Tyr Lys Leu Val Ser Met Lys Gly Thr Gly Val Phe Val Gln Asp
                485                 490                 495

Pro Ala Pro Ile Asp Ile Asp Ala Phe Pro Val Lys Pro Ile Cys Ser
            500                 505                 510

Ser Val Tyr Leu Gly Val Lys Gly Ser Gly His Tyr Gln Thr Asn Leu
        515                 520                 525

Tyr Ser Phe Asn Lys Ala Ile Asp Gly Phe Gly Val Phe Asp Ile Lys
    530                 535                 540

Asn Ser Ser Val Asn Thr Val Cys Phe Val Asp Val Asp Phe His Ser
545                 550                 555                 560

Val Glu Ile Glu Ala Gly Glu Val Lys Pro Phe Ala Val Tyr Lys Asn
                565                 570                 575

Val Lys Phe Tyr Leu Gly Asp Ile Ser His Leu Val Asn Cys Val Ser
            580                 585                 590

Phe Asp Phe Val Val Asn Ala Ala Asn Glu Asn Leu Leu His Gly Gly
        595                 600                 605

Gly Val Ala Arg Ala Ile Asp Ile Leu Thr Glu Gly Gln Leu Gln Ser
    610                 615                 620

Leu Ser Lys Asp Tyr Ile Ser Ser Asn Gly Pro Leu Lys Val Gly Ala
625                 630                 635                 640

Gly Val Met Leu Glu Cys Glu Lys Phe Asn Val Phe Asn Val Val Gly
                645                 650                 655

Pro Arg Thr Gly Lys His Glu His Ser Leu Leu Val Glu Ala Tyr Asn
            660                 665                 670

Ser Ile Leu Phe Glu Asn Gly Ile Pro Leu Met Pro Leu Leu Ser Cys
        675                 680                 685
```

```
Gly Ile Phe Gly Val Arg Ile Glu Asn Ser Leu Lys Ala Leu Phe Ser
    690                 695                 700

Cys Asp Ile Asn Lys Pro Leu Gln Val Phe Val Tyr Ser Ser Asn Glu
705                 710                 715                 720

Glu Gln Ala Val Leu Lys Phe Leu Asp Gly Leu Asp Leu Thr Pro Val
                725                 730                 735

Ile Asp Asp Val Asp Val Val Lys Pro Phe Arg Val Glu Gly Asn Phe
            740                 745                 750

Ser Phe Phe Asp Cys Gly Val Asn Ala Leu Asp Gly Asp Ile Tyr Leu
        755                 760                 765

Leu Phe Thr Asn Ser Ile Leu Met Leu Asp Lys Gln Gly Gln Leu Leu
    770                 775                 780

Asp Thr Lys Leu Asn Gly Ile Leu Gln Gln Ala Ala Leu Asp Tyr Leu
785                 790                 795                 800

Ala Thr Val Lys Thr Val Pro Ala Gly Asn Leu Val Lys Leu Phe Val
                805                 810                 815

Glu Ser Cys Thr Ile Tyr Met Cys Val Val Pro Ser Ile Asn Asp Leu
            820                 825                 830

Ser Phe Asp Lys Asn Leu Gly Arg Cys Val Arg Lys Leu Asn Arg Leu
        835                 840                 845

Lys Thr Cys Val Ile Ala Asn Val Pro Ala Ile Asp Val Leu Lys Lys
    850                 855                 860

Leu Leu Ser Ser Leu Thr Leu Thr Val Lys Phe Val Val Glu Ser Asn
865                 870                 875                 880

Val Met Asp Val Asn Asp Cys Phe Lys Asn Asp Asn Val Val Leu Lys
                885                 890                 895

Ile Thr Glu Asp Gly Ile Asn Val Lys Asp Val Val Val Glu Ser Ser
            900                 905                 910

Lys Ser Leu Gly Lys Gln Leu Gly Val Val Ser Asp Gly Val Asp Ser
```

915 920 925

Phe Glu Gly Val Leu Pro Ile Asn Thr Asp Thr Val Leu Ser Val Ala
930 935 940

Pro Glu Val Asp Trp Val Ala Phe Tyr Gly Phe Glu Lys Ala Ala Leu
945 950 955 960

Phe Ala Ser Leu Asp Val Lys Pro Tyr Gly Tyr Pro Asn Asp Phe Val
965 970 975

Gly Gly Phe Arg Val Leu Gly Thr Thr Asp Asn Asn Cys Trp Val Asn
980 985 990

Ala Thr Cys Ile Ile Leu Gln Tyr Leu Lys Pro Thr Phe Lys Ser Lys
995 1000 1005

Gly Leu Asn Val Leu Trp Asn Lys Phe Val Thr Gly Asp Val Gly
1010 1015 1020

Pro Phe Val Ser Phe Ile Tyr Phe Ile Thr Met Ser Ser Lys Gly
1025 1030 1035

Gln Lys Gly Asp Ala Glu Glu Ala Leu Ser Lys Leu Ser Glu Tyr
1040 1045 1050

Leu Ile Ser Asp Ser Ile Val Thr Leu Glu Gln Tyr Ser Thr Cys
1055 1060 1065

Asp Ile Cys Lys Ser Thr Val Val Glu Val Lys Ser Ala Ile Val
1070 1075 1080

Cys Ala Ser Val Leu Lys Asp Gly Cys Asp Val Gly Phe Cys Pro
1085 1090 1095

His Arg His Lys Leu Arg Ser Arg Val Lys Phe Val Asn Gly Arg
1100 1105 1110

Val Val Ile Thr Asn Val Gly Glu Pro Ile Ile Ser Gln Pro Ser
1115 1120 1125

Lys Leu Leu Asn Gly Ile Ala Tyr Thr Thr Phe Ser Gly Ser Phe
1130 1135 1140

```
Asp Asn  Gly His Tyr Val Val  Tyr Asp Ala Ala Asn  Asn Ala Val
    1145                1150                1155

Tyr Asp  Gly Ala Arg Leu Phe  Ser Ser Asp Leu Ser  Thr Leu Ala
    1160                1165                1170

Val Thr  Ala Ile Val Val Val  Gly Gly Cys Val Thr  Ser Asn Val
    1175                1180                1185

Pro Thr  Ile Val Ser Glu Lys  Ile Ser Val Met Asp  Lys Leu Asp
    1190                1195                1200

Thr Gly  Ala Gln Lys Phe Phe  Gln Phe Gly Asp Phe  Val Met Asn
    1205                1210                1215

Asn Ile  Val Leu Phe Leu Thr  Trp Leu Leu Ser Met  Phe Ser Leu
    1220                1225                1230

Leu Arg  Thr Ser Ile Met Lys  His Asp Ile Lys Val  Ile Ala Lys
    1235                1240                1245

Ala Pro  Lys Arg Thr Gly Val  Ile Leu Thr Arg Ser  Phe Lys Tyr
    1250                1255                1260

Asn Ile  Arg Ser Ala Leu Phe  Val Ile Lys Gln Lys  Trp Cys Val
    1265                1270                1275

Ile Val  Thr Leu Phe Lys Phe  Leu Leu Leu Leu Tyr  Ala Ile Tyr
    1280                1285                1290

Ala Leu  Val Phe Met Ile Val  Gln Phe Ser Pro Phe  Asn Ser Leu
    1295                1300                1305

Leu Cys  Gly Asp Ile Val Ser  Gly Tyr Glu Lys Ser  Thr Phe Asn
    1310                1315                1320

Lys Asp  Ile Tyr Cys Gly Asn  Ser Met Val Cys Lys  Met Cys Leu
    1325                1330                1335

Phe Ser  Tyr Gln Glu Phe Asn  Asp Leu Asp His Thr  Ser Leu Val
    1340                1345                1350
```

```
Trp Lys  His Ile Arg Asp Pro  Ile Leu Ile Ser Leu  Gln Pro Phe
    1355                1360                1365

Val Ile  Leu Val Ile Leu Leu  Ile Phe Gly Asn Met  Tyr Leu Arg
    1370                1375                1380

Phe Gly  Leu Leu Tyr Phe Val  Ala Gln Phe Ile Ser  Thr Phe Gly
    1385                1390                1395

Ser Phe  Leu Gly Phe His Gln  Lys Gln Trp Phe Leu  His Phe Val
    1400                1405                1410

Pro Phe  Asp Val Leu Cys Asn  Glu Phe Leu Ala Thr  Phe Ile Val
    1415                1420                1425

Cys Lys  Ile Val Leu Phe Val  Arg His Ile Ile Val  Gly Cys Asn
    1430                1435                1440

Asn Ala  Asp Cys Val Ala Cys  Ser Lys Ser Ala Arg  Leu Lys Arg
    1445                1450                1455

Val Pro  Leu Gln Thr Ile Ile  Asn Gly Met His Lys  Ser Phe Tyr
    1460                1465                1470

Val Asn  Ala Asn Gly Gly Thr  Cys Phe Cys Asn Lys  His Asn Phe
    1475                1480                1485

Phe Cys  Val Asn Cys Asp Ser  Phe Gly Pro Gly Asn  Thr Phe Ile
    1490                1495                1500

Asn Gly  Asp Ile Ala Arg Glu  Leu Gly Asn Val Val  Lys Thr Ala
    1505                1510                1515

Val Gln  Pro Thr Ala Pro Ala  Tyr Val Ile Ile Asp  Lys Val Asp
    1520                1525                1530

Phe Val  Asn Gly Phe Tyr Arg  Leu Tyr Ser Gly Asp  Thr Phe Trp
    1535                1540                1545

Arg Tyr  Asp Phe Asp Ile Thr  Glu Ser Lys Tyr Ser  Cys Lys Glu
    1550                1555                1560
```

```
Val Leu  Lys Asn Cys Asn Val  Leu Glu Asn Phe Ile  Val Tyr Asn
    1565                1570                1575

Asn Ser  Gly Ser Asn Ile Thr  Gln Ile Lys Asn Ala  Cys Val Tyr
    1580                1585                1590

Phe Ser  Gln Leu Leu Cys Glu  Pro Ile Lys Leu Val  Asn Ser Glu
    1595                1600                1605

Leu Leu  Ser Thr Leu Ser Val  Asp Phe Asn Gly Val  Leu His Lys
    1610                1615                1620

Ala Tyr  Val Asp Val Leu Cys  Asn Ser Phe Phe Lys  Glu Leu Thr
    1625                1630                1635

Ala Asn  Met Ser Met Ala Glu  Cys Lys Ala Thr Leu  Gly Leu Thr
    1640                1645                1650

Val Ser  Asp Asp Asp Phe Val  Ser Ala Val Ala Asn  Ala His Arg
    1655                1660                1665

Tyr Asp  Val Leu Leu Ser Asp  Leu Ser Phe Asn Asn  Phe Phe Ile
    1670                1675                1680

Ser Tyr  Ala Lys Pro Glu Asp  Lys Leu Ser Val Tyr  Asp Ile Ala
    1685                1690                1695

Cys Cys  Met Arg Ala Gly Ser  Lys Val Val Asn His  Asn Val Leu
    1700                1705                1710

Ile Lys  Glu Ser Ile Pro Ile  Val Trp Gly Val Lys  Asp Phe Asn
    1715                1720                1725

Thr Leu  Ser Gln Glu Gly Lys  Lys Tyr Leu Val Lys  Thr Thr Lys
    1730                1735                1740

Ala Lys  Gly Leu Thr Phe Leu  Leu Thr Phe Asn Asp  Asn Gln Ala
    1745                1750                1755

Ile Thr  Gln Val Pro Ala Thr  Ser Ile Val Ala Lys  Gln Gly Ala
    1760                1765                1770

Gly Phe  Lys Arg Thr Tyr Asn  Phe Leu Trp Tyr Val  Cys Leu Phe
```

1775 1780 1785

Val Val Ala Leu Phe Ile Gly Val Ser Phe Ile Asp Tyr Thr Thr
1790 1795 1800

Thr Val Thr Ser Phe His Gly Tyr Asp Phe Lys Tyr Ile Glu Asn
1805 1810 1815

Gly Gln Leu Lys Val Phe Glu Ala Pro Leu His Cys Val Arg Asn
1820 1825 1830

Val Phe Asp Asn Phe Asn Gln Trp His Glu Ala Lys Phe Gly Val
1835 1840 1845

Val Thr Thr Asn Ser Asp Lys Cys Pro Ile Val Val Gly Val Ser
1850 1855 1860

Glu Arg Ile Asn Val Val Pro Gly Val Pro Thr Asn Val Tyr Leu
1865 1870 1875

Val Gly Lys Thr Leu Val Phe Thr Leu Gln Ala Ala Phe Gly Asn
1880 1885 1890

Thr Gly Val Cys Tyr Asp Phe Asp Gly Val Thr Thr Ser Asp Lys
1895 1900 1905

Cys Ile Phe Asn Ser Ala Cys Thr Arg Leu Glu Gly Leu Gly Gly
1910 1915 1920

Asp Asn Val Tyr Cys Tyr Asn Thr Asp Leu Ile Glu Gly Ser Lys
1925 1930 1935

Pro Tyr Ser Thr Leu Gln Pro Asn Ala Tyr Tyr Lys Tyr Asp Ala
1940 1945 1950

Lys Asn Tyr Val Arg Phe Pro Glu Ile Leu Ala Arg Gly Phe Gly
1955 1960 1965

Leu Arg Thr Ile Arg Thr Leu Ala Thr Arg Tyr Cys Arg Val Gly
1970 1975 1980

Glu Cys Arg Asp Ser His Lys Gly Val Cys Phe Gly Phe Asp Lys
1985 1990 1995

```
Trp Tyr  Val Asn Asp Gly Arg  Val Asp Asp Gly Tyr  Ile Cys Gly
    2000                 2005                 2010

Asp Gly  Leu Ile Asp Leu Leu  Val Asn Val Leu Ser  Ile Phe Ser
    2015                 2020                 2025

Ser Ser  Phe Ser Val Val Ala  Met Ser Gly His Met  Leu Phe Asn
    2030                 2035                 2040

Phe Leu  Phe Ala Ala Phe Ile  Thr Phe Leu Cys Phe  Leu Val Thr
    2045                 2050                 2055

Lys Phe  Lys Arg Val Phe Gly  Asp Leu Ser Tyr Gly  Val Phe Thr
    2060                 2065                 2070

Val Val  Cys Ala Thr Leu Ile  Asn Asn Ile Ser Tyr  Val Val Thr
    2075                 2080                 2085

Gln Asn  Leu Phe Phe Met Leu  Leu Tyr Ala Ile Leu  Tyr Phe Val
    2090                 2095                 2100

Phe Thr  Arg Thr Val Arg Tyr  Ala Trp Ile Trp His  Ile Ala Tyr
    2105                 2110                 2115

Ile Val  Ala Tyr Phe Leu Leu  Ile Pro Trp Trp Leu  Leu Thr Trp
    2120                 2125                 2130

Phe Ser  Phe Ala Ala Phe Leu  Glu Leu Leu Pro Asn  Val Phe Lys
    2135                 2140                 2145

Leu Lys  Ile Ser Thr Gln Leu  Phe Glu Gly Asp Lys  Phe Ile Gly
    2150                 2155                 2160

Thr Phe  Glu Ser Ala Ala Ala  Gly Thr Phe Val Leu  Asp Met Arg
    2165                 2170                 2175

Ser Tyr  Glu Arg Leu Ile Asn  Thr Ile Ser Pro Glu  Lys Leu Lys
    2180                 2185                 2190

Asn Tyr  Ala Ala Ser Tyr Asn  Lys Tyr Lys Tyr Tyr  Ser Gly Ser
    2195                 2200                 2205
```

```
Ala Ser  Glu Ala Asp Tyr Arg  Cys Ala Cys Tyr Ala  His Leu Ala
    2210                 2215                 2220

Lys Ala  Met Leu Asp Tyr Ala  Lys Asp His Asn Asp  Met Leu Tyr
    2225                 2230                 2235

Ser Pro  Pro Thr Ile Ser Tyr  Asn Ser Thr Leu Gln
    2240                 2245                 2250
```

<210> 59
<211> 361
<212> PRT
<213> Human coronavirus

<220>
<221> MISC_FEATURE
<222> (1)..(361)
<223> 3CI pro Coronavirus polyprotein processing endoprotease

<400> 59

```
Ser Gly Leu Lys Lys Met Ala Gln Pro Ser Gly Cys Val Glu Arg Cys
1               5                  10                  15

Val Val Arg Val Cys Tyr Gly Ser Thr Val Leu Asn Gly Val Trp Leu
            20                  25                  30

Gly Asp Thr Val Thr Cys Pro Arg His Val Ile Ala Pro Ser Thr Thr
        35                  40                  45

Val Leu Ile Asp Tyr Asp His Ala Tyr Ser Thr Met Arg Leu His Asn
    50                  55                  60

Phe Ser Val Ser His Asn Gly Val Phe Leu Gly Val Val Gly Val Thr
65                  70                  75                  80

Met His Gly Ser Val Leu Arg Ile Lys Val Ser Gln Ser Asn Val His
                85                  90                  95

Thr Pro Lys His Val Phe Lys Thr Leu Lys Pro Gly Asp Ser Phe Asn
            100                 105                 110

Ile Leu Ala Cys Tyr Glu Gly Ile Ala Ser Gly Val Phe Gly Val Asn
        115                 120                 125
```

```
Leu Arg Thr Asn Phe Thr Ile Lys Gly Ser Phe Ile Asn Gly Ala Cys
    130                 135                 140

Gly Ser Pro Gly Tyr Asn Val Arg Asn Asp Gly Thr Val Glu Phe Cys
145                 150                 155                 160

Tyr Leu His Gln Ile Glu Leu Gly Ser Gly Ala His Val Gly Ser Asp
                165                 170                 175

Phe Thr Gly Ser Val Tyr Gly Asn Phe Asp Asp Gln Pro Ser Leu Gln
            180                 185                 190

Val Glu Ser Ala Asn Leu Met Leu Ser Asp Asn Val Val Ala Phe Leu
        195                 200                 205

Tyr Ala Ala Leu Leu Asn Gly Cys Arg Trp Trp Leu Cys Ser Thr Arg
    210                 215                 220

Val Asn Val Asp Gly Phe Asn Glu Trp Ala Met Ala Asn Gly Tyr Thr
225                 230                 235                 240

Ser Val Ser Ser Val Glu Cys Tyr Ser Ile Leu Ala Ala Lys Thr Gly
                245                 250                 255

Val Ser Val Glu Gln Leu Leu Ala Ser Ile Gln His Leu His Glu Gly
            260                 265                 270

Phe Gly Gly Lys Asn Ile Leu Gly Tyr Ser Ser Leu Cys Asp Glu Phe
        275                 280                 285

Thr Leu Ala Glu Val Val Lys Gln Met Tyr Gly Val Asn Leu Gln Ser
    290                 295                 300

Gly Lys Val Ile Phe Gly Leu Lys Thr Met Phe Leu Phe Ser Val Phe
305                 310                 315                 320

Phe Thr Met Phe Trp Ala Glu Leu Phe Ile Tyr Thr Asn Thr Ile Trp
                325                 330                 335

Ile Asn Pro Val Ile Leu Thr Pro Ile Phe Cys Leu Leu Leu Phe Leu
            340                 345                 350
```

Ser Leu Val Leu Thr Met Phe Leu Lys
355 360

<210> 60
<211> 1071
<212> PRT
<213> Human coronavirus

<220>
<221> MISC_FEATURE
<222> (1)..(1071)
<223> RNA dependant RNA polymerase (pfam00680)

<400> 60

Ala Gly Lys Gln Thr Glu Leu Ala Val Asn Ser Gly Leu Leu Thr Ala
1 5 10 15

Cys Ala Phe Ser Val Asp Pro Ala Thr Thr Tyr Leu Glu Ala Val Lys
20 25 30

His Gly Ala Lys Pro Val Ser Asn Cys Ile Lys Met Leu Ser Asn Gly
35 40 45

Ala Gly Asn Gly Gln Ala Ile Thr Thr Ser Val Asp Ala Asn Thr Asn
50 55 60

Gln Asp Ser Tyr Gly Gly Ala Ser Ile Cys Leu Tyr Cys Arg Ala His
65 70 75 80

Val Pro His Pro Ser Met Asp Gly Tyr Cys Lys Phe Lys Gly Lys Cys
85 90 95

Val Gln Val Pro Ile Gly Cys Leu Asp Pro Ile Arg Phe Cys Leu Glu
100 105 110

Asn Asn Val Cys Asn Val Cys Gly Cys Trp Leu Gly His Gly Cys Ala
115 120 125

Cys Asp Arg Thr Thr Ile Gln Ser Val Asp Ile Ser Tyr Leu Asn Glu
130 135 140

Gln Gly Val Leu Val Gln Leu Asp Arg Ala Arg Gly Ser Ser Ala Ala
145 150 155 160

```
Arg Leu Glu Pro Cys Asn Gly Thr Asp Ile Asp Lys Cys Val Arg Ala
                165                 170                 175

Phe Asp Ile Tyr Asn Lys Asn Val Ser Phe Leu Gly Lys Cys Leu Lys
            180                 185                 190

Met Asn Cys Val Arg Phe Lys Asn Ala Asp Leu Lys Asp Gly Tyr Phe
        195                 200                 205

Val Ile Lys Arg Cys Thr Lys Ser Val Met Glu His Glu Gln Ser Met
    210                 215                 220

Tyr Asn Leu Leu Asn Phe Ser Gly Ala Leu Ala Glu His Asp Phe Phe
225                 230                 235                 240

Thr Trp Lys Asp Gly Arg Val Ile Tyr Gly Asn Val Ser Arg His Asn
                245                 250                 255

Leu Thr Lys Tyr Thr Met Met Asp Leu Val Tyr Ala Met Arg Asn Phe
            260                 265                 270

Asp Glu Gln Asn Cys Asp Val Leu Lys Glu Val Leu Val Leu Thr Gly
        275                 280                 285

Cys Cys Asp Asn Ser Tyr Phe Asp Ser Lys Gly Trp Tyr Asp Pro Val
    290                 295                 300

Glu Asn Glu Asp Ile His Arg Val Tyr Ala Ser Leu Gly Lys Ile Val
305                 310                 315                 320

Ala Arg Ala Met Leu Lys Cys Val Ala Leu Cys Asp Ala Met Val Ala
                325                 330                 335

Lys Gly Val Val Gly Val Leu Thr Leu Asp Asn Gln Asp Leu Asn Gly
            340                 345                 350

Asn Phe Tyr Asp Phe Gly Asp Phe Val Val Ser Leu Pro Asn Met Gly
        355                 360                 365

Val Pro Cys Cys Thr Ser Tyr Tyr Ser Tyr Met Met Pro Ile Met Gly
    370                 375                 380

Leu Thr Asn Cys Leu Ala Ser Glu Cys Phe Val Lys Ser Asp Ile Phe
```

```
385                 390                 395                 400

Gly Ser Asp Phe Lys Thr Phe Asp Leu Leu Lys Tyr Asp Phe Thr Glu
                405                 410                 415

His Lys Glu Asn Leu Phe Asn Lys Tyr Phe Lys His Trp Ser Phe Asp
            420                 425                 430

Tyr His Pro Asn Cys Cys Asp Cys Tyr Asp Asp Met Cys Val Ile His
        435                 440                 445

Cys Ala Asn Phe Asn Thr Leu Phe Ala Thr Thr Ile Pro Gly Thr Ala
    450                 455                 460

Phe Gly Pro Leu Cys Arg Lys Val Phe Ile Asp Gly Val Pro Leu Val
465                 470                 475                 480

Thr Thr Ala Gly Tyr His Phe Lys Gln Leu Gly Leu Val Trp Asn Lys
                485                 490                 495

Asp Val Asn Thr His Ser Val Arg Leu Thr Ile Thr Glu Leu Leu Gln
            500                 505                 510

Phe Val Thr Asp Pro Ser Leu Ile Ile Ala Ser Ser Pro Ala Leu Val
        515                 520                 525

Asp Gln Arg Thr Ile Cys Phe Ser Val Ala Ala Leu Ser Thr Gly Leu
    530                 535                 540

Thr Asn Gln Val Val Lys Pro Gly His Phe Asn Glu Glu Phe Tyr Asn
545                 550                 555                 560

Phe Leu Arg Leu Arg Gly Phe Phe Asp Glu Gly Ser Glu Leu Thr Leu
                565                 570                 575

Lys His Phe Phe Phe Ala Gln Asn Gly Asp Ala Ala Val Lys Asp Phe
            580                 585                 590

Asp Phe Tyr Arg Tyr Asn Lys Pro Thr Ile Leu Asp Ile Cys Gln Ala
        595                 600                 605

Arg Val Thr Tyr Lys Ile Val Ser Arg Tyr Phe Asp Ile Tyr Glu Gly
    610                 615                 620
```

```
Gly Cys Ile Lys Ala Cys Glu Val Val Val Thr Asn Leu Asn Lys Ser
625                 630                 635                 640

Ala Gly Trp Pro Leu Asn Lys Phe Gly Lys Ala Ser Leu Tyr Tyr Glu
                645                 650                 655

Ser Ile Ser Tyr Glu Glu Gln Asp Ala Leu Phe Ala Leu Thr Lys Arg
            660                 665                 670

Asn Val Leu Pro Thr Met Thr Gln Leu Asn Leu Lys Tyr Ala Ile Ser
        675                 680                 685

Gly Lys Glu Arg Ala Arg Thr Val Gly Gly Val Ser Leu Leu Ser Thr
    690                 695                 700

Met Thr Thr Arg Gln Tyr His Gln Lys His Leu Lys Ser Ile Val Asn
705                 710                 715                 720

Thr Arg Asn Ala Thr Val Val Ile Gly Thr Thr Lys Phe Tyr Gly Gly
                725                 730                 735

Trp Asn Asn Met Leu Arg Thr Leu Ile Asp Gly Val Glu Asn Pro Met
            740                 745                 750

Leu Met Gly Trp Asp Tyr Pro Lys Cys Asp Arg Ala Leu Pro Asn Met
        755                 760                 765

Ile Arg Met Ile Ser Ala Met Val Leu Gly Ser Lys His Val Asn Cys
    770                 775                 780

Cys Thr Ala Thr Asp Arg Phe Tyr Arg Leu Gly Asn Glu Leu Ala Gln
785                 790                 795                 800

Val Leu Thr Glu Val Val Tyr Ser Asn Gly Gly Phe Tyr Phe Lys Pro
                805                 810                 815

Gly Gly Thr Thr Ser Gly Asp Ala Ser Thr Ala Tyr Ala Asn Ser Ile
            820                 825                 830

Phe Asn Ile Phe Gln Ala Val Ser Ser Asn Ile Asn Arg Leu Leu Ser
        835                 840                 845
```

```
Val Pro Ser Asp Ser Cys Asn Asn Val Asn Val Arg Asp Leu Gln Arg
    850                 855                 860

Arg Leu Tyr Asp Asn Cys Tyr Arg Leu Thr Ser Val Glu Glu Ser Phe
865                 870                 875                 880

Ile Glu Asp Tyr Tyr Gly Tyr Leu Arg Lys His Phe Ser Met Met Ile
                885                 890                 895

Leu Ser Asp Asp Gly Val Val Cys Tyr Asn Lys Asp Tyr Ala Glu Leu
            900                 905                 910

Gly Tyr Ile Ala Asp Ile Ser Ala Phe Lys Ala Thr Leu Tyr Tyr Gln
        915                 920                 925

Asn Asn Val Phe Met Ser Thr Ser Lys Cys Trp Val Glu Glu Asp Leu
    930                 935                 940

Thr Lys Gly Pro His Glu Phe Cys Ser Gln His Thr Met Gln Ile Val
945                 950                 955                 960

Asp Lys Asp Gly Thr Tyr Tyr Leu Pro Tyr Pro Asp Pro Ser Arg Ile
                965                 970                 975

Leu Ser Ala Gly Val Phe Val Asp Asp Val Val Lys Thr Asp Ala Val
            980                 985                 990

Val Leu Leu Glu Arg Tyr Val Ser  Leu Ala Ile Asp Ala  Tyr Pro Leu
        995                 1000                1005

Ser Lys  His Pro Asn Ser Glu  Tyr Arg Lys Val Phe  Tyr Val Leu
    1010                1015                1020

Leu Asp  Trp Val Lys His Leu  Asn Lys Asn Leu Asn  Glu Gly Val
    1025                1030                1035

Leu Glu  Ser Phe Ser Val Thr  Leu Leu Asp Asn Gln  Glu Asp Lys
    1040                1045                1050

Phe Trp  Cys Glu Asp Phe Tyr  Ala Ser Met Tyr Glu  Asn Ser Thr
    1055                1060                1065
```

Ile Leu Gln
1070

<210> 61
<211> 1115
<212> PRT
<213> Human coronavirus

<220>
<221> MISC_FEATURE
<222> (1)..(1115)
<223> ExoN 3' to 5' Exonuclease and helicase

<400> 61

Ala Ala Gly Leu Cys Val Val Cys Gly Ser Gln Thr Val Leu Arg Cys
1 5 10 15

Gly Asp Cys Leu Arg Lys Pro Met Leu Cys Thr Lys Cys Ala Tyr Asp
20 25 30

His Val Phe Gly Thr Asp His Lys Phe Ile Leu Ala Ile Thr Pro Tyr
35 40 45

Val Cys Asn Ala Ser Gly Cys Gly Val Ser Asp Val Lys Lys Leu Tyr
50 55 60

Leu Gly Gly Leu Asn Tyr Tyr Cys Thr Asn His Lys Pro Gln Leu Ser
65 70 75 80

Phe Pro Leu Cys Ser Ala Gly Asn Ile Phe Gly Leu Tyr Lys Asn Ser
85 90 95

Ala Thr Gly Ser Leu Asp Val Glu Val Phe Asn Arg Leu Ala Thr Ser
100 105 110

Asp Trp Thr Asp Val Arg Asp Tyr Lys Leu Ala Asn Asp Val Lys Asp
115 120 125

Thr Leu Arg Leu Phe Ala Ala Glu Thr Ile Lys Ala Lys Glu Glu Ser
130 135 140

Val Lys Ser Ser Tyr Ala Phe Ala Thr Leu Lys Glu Val Val Gly Pro
145 150 155 160

```
Lys Glu Leu Leu Leu Ser Trp Glu Ser Gly Lys Val Lys Pro Pro Leu
                165                 170                 175

Asn Arg Asn Ser Val Phe Thr Cys Phe Gln Ile Ser Lys Asp Ser Lys
            180                 185                 190

Phe Gln Ile Gly Glu Phe Ile Phe Glu Lys Val Glu Tyr Gly Ser Asp
        195                 200                 205

Thr Val Thr Tyr Lys Ser Thr Val Thr Thr Lys Leu Val Pro Gly Met
    210                 215                 220

Ile Phe Val Leu Thr Ser His Asn Val Gln Pro Leu Arg Ala Pro Thr
225                 230                 235                 240

Ile Ala Asn Gln Glu Lys Tyr Ser Ser Ile Tyr Lys Leu His Pro Ala
                245                 250                 255

Phe Asn Val Ser Asp Ala Tyr Ala Asn Leu Val Pro Tyr Tyr Gln Leu
            260                 265                 270

Ile Gly Lys Gln Lys Ile Thr Thr Ile Gln Gly Pro Pro Gly Ser Gly
        275                 280                 285

Lys Ser His Cys Ser Ile Gly Leu Gly Leu Tyr Tyr Pro Gly Ala Arg
    290                 295                 300

Ile Val Phe Val Ala Cys Ala His Ala Ala Val Asp Ser Leu Cys Ala
305                 310                 315                 320

Lys Ala Met Thr Val Tyr Ser Ile Asp Lys Cys Thr Arg Ile Ile Pro
                325                 330                 335

Ala Arg Ala Arg Val Glu Cys Tyr Ser Gly Phe Lys Pro Asn Asn Thr
            340                 345                 350

Ser Ala Gln Tyr Ile Phe Ser Thr Val Asn Ala Leu Pro Glu Cys Asn
        355                 360                 365

Ala Asp Ile Val Val Val Asp Glu Val Ser Met Cys Thr Asn Tyr Asp
    370                 375                 380

Leu Ser Val Ile Asn Gln Arg Leu Ser Tyr Lys His Ile Val Tyr Val
```

```
385                 390                 395                 400

Gly Asp Pro Gln Gln Leu Pro Ala Pro Arg Val Met Ile Thr Lys Gly
                405                 410                 415

Val Met Glu Pro Val Asp Tyr Asn Val Val Thr Gln Arg Met Cys Ala
            420                 425                 430

Ile Gly Pro Asp Val Phe Leu His Lys Cys Tyr Arg Cys Pro Ala Glu
        435                 440                 445

Ile Val Ile Gln Phe Leu Asn Leu Phe Met Arg Thr Ser Leu Ser Leu
    450                 455                 460

Leu Asn Leu Leu Val Asn Ser Val Leu Lys Ser Phe Leu Arg Val Met
465                 470                 475                 480

Tyr Lys Val Asp Asn Gly Ser Ser Ile Asn Arg Lys Gln Leu Glu Ile
                485                 490                 495

Val Lys Leu Phe Leu Val Lys Asn Pro Ser Trp Ser Lys Ala Val Phe
            500                 505                 510

Ile Ser Pro Tyr Asn Ser Gln Asn Tyr Val Ala Ser Arg Phe Leu Gly
        515                 520                 525

Leu Gln Ile Gln Thr Val Asp Ser Ser Gln Gly Ser Glu Tyr Asp Tyr
    530                 535                 540

Val Ile Tyr Ala Gln Thr Ser Asp Thr Ala His Ala Cys Asn Val Asn
545                 550                 555                 560

Arg Phe Asn Val Ala Ile Thr Arg Ala Lys Lys Gly Ile Phe Cys Val
                565                 570                 575

Met Cys Asp Lys Thr Leu Phe Asp Ser Leu Lys Phe Phe Glu Ile Lys
            580                 585                 590

His Ala Asp Leu His Ser Ser Gln Val Cys Gly Leu Phe Lys Asn Cys
        595                 600                 605

Thr Arg Thr Pro Leu Asn Leu Pro Pro Thr His Ala His Thr Phe Leu
    610                 615                 620
```

```
Ser Leu Ser Asp Gln Phe Lys Thr Thr Gly Asp Leu Ala Val Gln Ile
625                 630                 635                 640

Gly Ser Asn Asn Val Cys Thr Tyr Glu His Val Ile Ser Phe Met Gly
                645                 650                 655

Phe Arg Phe Asp Ile Ser Ile Pro Gly Ser His Ser Leu Phe Cys Thr
            660                 665                 670

Arg Asp Phe Ala Ile Arg Asn Val Arg Gly Trp Leu Gly Met Asp Val
        675                 680                 685

Glu Ser Ala His Val Cys Gly Asp Asn Ile Gly Thr Asn Val Pro Leu
    690                 695                 700

Gln Val Gly Phe Ser Asn Gly Val Asn Phe Val Val Gln Thr Glu Gly
705                 710                 715                 720

Cys Val Ser Thr Asn Phe Gly Asp Val Ile Lys Pro Val Cys Ala Lys
                725                 730                 735

Ser Pro Pro Gly Glu Gln Phe Arg His Leu Ile Pro Leu Leu Arg Lys
            740                 745                 750

Gly Gln Pro Trp Leu Ile Val Arg Arg Arg Ile Val Gln Met Ile Ser
        755                 760                 765

Asp Tyr Leu Ser Asn Leu Ser Asp Ile Leu Val Phe Val Leu Trp Ala
    770                 775                 780

Gly Ser Leu Glu Leu Thr Thr Met Arg Tyr Phe Val Lys Ile Gly Pro
785                 790                 795                 800

Ile Lys Tyr Cys Tyr Cys Gly Asn Phe Ala Thr Cys Tyr Asn Ser Val
                805                 810                 815

Ser Asn Glu Tyr Cys Cys Phe Lys His Ala Leu Gly Cys Asp Tyr Val
            820                 825                 830

Tyr Asn Pro Tyr Ala Phe Asp Ile Gln Gln Trp Gly Tyr Val Gly Ser
        835                 840                 845
```

```
Leu Ser Gln Asn His His Thr Phe Cys Asn Ile His Arg Asn Glu His
    850                 855                 860

Asp Ala Ser Gly Asp Ala Val Met Thr Arg Cys Leu Ala Val His Asp
865                 870                 875                 880

Cys Phe Val Lys Asn Val Asp Trp Thr Val Thr Tyr Pro Phe Ile Ala
                885                 890                 895

Asn Glu Lys Phe Ile Asn Gly Cys Gly Arg Asn Val Gln Gly His Val
            900                 905                 910

Val Arg Ala Ala Leu Lys Leu Tyr Lys Pro Ser Val Ile His Asp Ile
        915                 920                 925

Gly Asn Pro Lys Gly Val Arg Cys Ala Val Thr Asp Ala Lys Trp Tyr
    930                 935                 940

Cys Tyr Asp Lys Gln Pro Val Asn Ser Asn Val Lys Leu Leu Asp Tyr
945                 950                 955                 960

Asp Tyr Ala Thr His Gly Gln Leu Asp Gly Leu Cys Leu Phe Trp Asn
                965                 970                 975

Cys Asn Val Asp Met Tyr Pro Glu Phe Ser Ile Val Cys Arg Phe Asp
            980                 985                 990

Thr Arg Thr Arg Ser Val Phe Asn  Leu Glu Gly Val Asn  Gly Gly Ser
        995                 1000                1005

Leu Tyr  Val Asn Lys His Ala  Phe His Thr Pro Ala  Tyr Asp Lys
    1010                1015                1020

Arg Ala  Phe Val Lys Leu Lys  Pro Met Pro Phe Phe  Tyr Phe Asp
    1025                1030                1035

Asp Ser  Asp Cys Asp Val Val  Gln Glu Gln Val Asn  Tyr Val Pro
    1040                1045                1050

Leu Arg  Ala Ser Ser Cys Val  Thr Arg Cys Asn Ile  Gly Gly Ala
    1055                1060                1065
```

```
Val Cys  Ser Lys His Ala Asn  Leu Tyr Gln Lys Tyr  Val Glu Ala
    1070                1075                1080

Tyr Asn  Thr Phe Thr Gln Ala  Gly Phe Asn Ile Trp  Val Pro His
    1085                1090                1095

Ser Phe  Asp Val Tyr Asn Leu  Trp Gln Ile Phe Ile  Glu Thr Asn
    1100                1105                1110

Leu Gln
    1115
```

<210> 62
<211> 344
<212> PRT
<213> Human coronavirus

<220>
<221> MISC_FEATURE
<222> (1)..(344)
<223> XendoU (homolog of) polyU-specific endoribonuclease

<400> 62

```
Ser Leu Glu Asn Ile Ala Phe Asn Val Val Lys Lys Gly Cys Phe Thr
1               5                   10                  15

Gly Val Asp Gly Glu Leu Pro Val Ala Val Val Asn Asp Lys Val Phe
            20                  25                  30

Val Arg Tyr Gly Asp Val Asp Asn Leu Val Phe Thr Asn Lys Thr Thr
        35                  40                  45

Leu Pro Thr Asn Val Ala Phe Glu Leu Phe Ala Lys Arg Lys Met Gly
    50                  55                  60

Leu Thr Pro Pro Leu Ser Ile Leu Lys Asn Leu Gly Val Val Ala Thr
65                  70                  75                  80

Tyr Lys Phe Val Leu Trp Asp Tyr Glu Ala Glu Arg Pro Phe Thr Ser
                85                  90                  95

Tyr Thr Lys Ser Val Cys Lys Tyr Thr Asp Phe Asn Glu Asp Val Cys
            100                 105                 110
```

```
Val Cys Phe Asp Asn Ser Ile Gln Gly Ser Tyr Glu Arg Phe Thr Leu
        115                 120                 125

Thr Thr Asn Ala Val Leu Phe Ser Thr Val Val Ile Lys Asn Leu Thr
    130                 135                 140

Pro Ile Lys Leu Asn Phe Gly Met Leu Asn Gly Met Pro Val Ser Ser
145                 150                 155                 160

Ile Lys Gly Asp Lys Gly Val Glu Lys Leu Val Asn Trp Tyr Ile Tyr
                165                 170                 175

Val Arg Lys Asn Gly Gln Phe Gln Asp His Tyr Asp Gly Phe Tyr Thr
            180                 185                 190

Gln Gly Arg Asn Leu Ser Asp Phe Thr Pro Arg Ser Asp Met Glu Tyr
        195                 200                 205

Asp Phe Leu Asn Met Asp Met Gly Val Phe Ile Asn Lys Tyr Gly Leu
    210                 215                 220

Glu Asp Phe Asn Phe Glu His Val Val Tyr Gly Asp Val Ser Lys Thr
225                 230                 235                 240

Thr Leu Gly Gly Leu His Leu Leu Ile Ser Gln Phe Arg Leu Ser Lys
                245                 250                 255

Met Gly Val Leu Lys Ala Asp Asp Phe Val Thr Ala Ser Asp Thr Thr
            260                 265                 270

Leu Arg Cys Cys Thr Val Thr Tyr Leu Asn Glu Leu Ser Ser Lys Val
        275                 280                 285

Val Cys Thr Tyr Met Asp Leu Leu Leu Asp Asp Phe Val Thr Ile Leu
    290                 295                 300

Lys Ser Leu Asp Leu Gly Val Ile Ser Lys Val His Glu Val Ile Ile
305                 310                 315                 320

Asp Asn Lys Pro Tyr Arg Trp Met Leu Trp Cys Lys Asp Asn His Leu
                325                 330                 335

Ser Thr Phe Tyr Pro Gln Leu Gln
```

340

<210> 63
<211> 300
<212> PRT
<213> Human coronavirus

<220>
<221> MISC_FEATURE
<222> (1)..(300)
<223> 2'-O-MT 2: S-adenosylmethionine-dependant ribose 2'-orthomethyl
ansferase

<400> 63

Ser Ala Glu Trp Lys Cys Gly Tyr Ala Met Pro Gln Ile Tyr Lys Leu
1 5 10 15

Gln Arg Met Cys Leu Glu Pro Cys Asn Leu Tyr Asn Tyr Gly Ala Gly
20 25 30

Ile Lys Leu Pro Ser Gly Ile Met Leu Asn Val Val Lys Tyr Thr Gln
35 40 45

Leu Cys Gln Tyr Leu Asn Ser Thr Thr Met Cys Val Pro His Asn Met
50 55 60

Arg Val Leu His Tyr Gly Ala Gly Ser Asp Lys Gly Val Ala Pro Gly
65 70 75 80

Thr Thr Val Leu Lys Arg Trp Leu Pro Pro Asp Ala Ile Ile Ile Asp
85 90 95

Asn Asp Ile Asn Asp Tyr Val Ser Asp Ala Asp Phe Ser Ile Thr Gly
100 105 110

Asp Cys Ala Thr Val Tyr Leu Glu Asp Lys Phe Asp Leu Leu Ile Ser
115 120 125

Asp Met Tyr Asp Gly Arg Ile Lys Phe Cys Asp Gly Glu Asn Val Ser
130 135 140

Lys Asp Gly Phe Phe Thr Tyr Leu Asn Gly Val Ile Arg Glu Lys Leu
145 150 155 160

```
Ala Ile Gly Gly Ser Val Ala Ile Lys Ile Thr Glu Tyr Ser Trp Asn
                165                 170                 175

Lys Tyr Leu Tyr Glu Leu Ile Gln Arg Phe Ala Phe Trp Thr Leu Phe
            180                 185                 190

Cys Thr Ser Val Asn Thr Ser Ser Ser Glu Ala Phe Leu Ile Gly Ile
        195                 200                 205

Asn Tyr Leu Gly Asp Phe Ile Gln Gly Pro Phe Ile Ala Gly Asn Thr
    210                 215                 220

Val His Ala Asn Tyr Ile Phe Trp Arg Asn Ser Thr Ile Met Ser Leu
225                 230                 235                 240

Ser Tyr Asn Ser Val Leu Asp Leu Ser Lys Phe Glu Cys Lys His Lys
                245                 250                 255

Ala Thr Val Val Val Thr Leu Lys Asp Ser Asp Val Asn Asp Met Val
            260                 265                 270

Leu Ser Leu Ile Lys Ser Gly Arg Leu Leu Leu Arg Asn Asn Gly Arg
        275                 280                 285

Phe Gly Gly Phe Ser Asn His Leu Val Ser Thr Lys
    290                 295                 300
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<210> 64
<211> 1356
<212> PRT
<213> Human coronavirus

<220>
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<223> ORF-2 Spike protein/S-gene

<400> 64

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1               5                   10                  15

Phe Thr Cys Asn Ser Asn Ala Asn Leu Ser Met Leu Gln Leu Gly Val
            20                  25                  30
```

```
Pro Asp Asn Ser Ser Thr Ile Val Thr Gly Leu Leu Pro Thr His Trp
        35                  40                  45

Phe Cys Ala Asn Gln Ser Thr Ser Val Tyr Ser Ala Asn Gly Phe Phe
    50                  55                  60

Tyr Ile Asp Val Gly Asn His Arg Ser Ala Phe Ala Leu His Thr Gly
65                  70                  75                  80

Tyr Tyr Asp Ala Asn Gln Tyr Tyr Ile Tyr Val Thr Asn Glu Ile Gly
                85                  90                  95

Leu Asn Ala Ser Val Thr Leu Lys Ile Cys Lys Phe Ser Arg Asn Thr
            100                 105                 110

Thr Phe Asp Phe Leu Ser Asn Ala Ser Ser Ser Phe Asp Cys Ile Val
        115                 120                 125

Asn Leu Leu Phe Thr Glu Gln Leu Gly Ala Pro Leu Gly Ile Thr Ile
    130                 135                 140

Ser Gly Glu Thr Val Arg Leu His Leu Tyr Asn Val Thr Arg Thr Phe
145                 150                 155                 160

Tyr Val Pro Ala Ala Tyr Lys Leu Thr Lys Leu Ser Val Lys Cys Tyr
                165                 170                 175

Phe Asn Tyr Ser Cys Val Phe Ser Val Val Asn Ala Thr Val Thr Val
            180                 185                 190

Asn Val Thr Thr His Asn Gly Arg Val Val Asn Tyr Thr Val Cys Asp
        195                 200                 205

Asp Cys Asn Gly Tyr Thr Asp Asn Ile Phe Ser Val Gln Gln Asp Gly
    210                 215                 220

Arg Ile Pro Asn Gly Phe Pro Phe Asn Asn Trp Phe Leu Leu Thr Asn
225                 230                 235                 240

Gly Ser Thr Leu Val Asp Gly Val Ser Arg Leu Tyr Gln Pro Leu Arg
                245                 250                 255

Leu Thr Cys Leu Trp Pro Val Pro Gly Leu Lys Ser Ser Thr Gly Phe
```

260 265 270

Val Tyr Phe Asn Ala Thr Gly Ser Asp Val Asn Cys Asn Gly Tyr Gln
275 280 285

His Asn Ser Val Val Asp Val Met Arg Tyr Asn Leu Asn Phe Ser Ala
290 295 300

Asn Ser Leu Asp Asn Leu Lys Ser Gly Val Ile Val Phe Lys Thr Leu
305 310 315 320

Gln Tyr Asp Val Leu Phe Tyr Cys Ser Asn Ser Ser Ser Gly Val Leu
325 330 335

Asp Thr Thr Ile Pro Phe Gly Pro Ser Ser Gln Pro Tyr Tyr Cys Phe
340 345 350

Ile Asn Ser Thr Ile Asn Thr Thr His Val Ser Thr Phe Val Gly Ile
355 360 365

Leu Pro Pro Thr Val Arg Glu Ile Val Val Ala Arg Thr Gly Gln Phe
370 375 380

Tyr Ile Asn Gly Phe Lys Tyr Phe Asp Leu Gly Phe Ile Glu Ala Val
385 390 395 400

Asn Phe Asn Val Thr Thr Ala Ser Ala Thr Asp Phe Trp Thr Val Ala
405 410 415

Phe Ala Thr Phe Val Asp Val Leu Val Asn Val Ser Ala Thr Asn Ile
420 425 430

Gln Asn Leu Leu Tyr Cys Asp Ser Pro Phe Glu Lys Leu Gln Cys Glu
435 440 445

His Leu Gln Phe Gly Leu Gln Asp Gly Phe Tyr Ser Ala Asn Phe Leu
450 455 460

Asp Asp Asn Val Leu Pro Glu Thr Tyr Val Ala Leu Pro Ile Tyr Tyr
465 470 475 480

Gln His Thr Asp Ile Asn Phe Thr Ala Thr Ala Ser Phe Gly Gly Ser
485 490 495

```
Cys Tyr Val Cys Lys Pro His Gln Val Asn Ile Ser Leu Asn Gly Asn
            500                 505                 510

Thr Ser Val Cys Val Arg Thr Ser His Phe Ser Ile Arg Tyr Ile Tyr
        515                 520                 525

Asn Arg Val Lys Ser Gly Ser Pro Gly Asp Ser Ser Trp His Ile Tyr
    530                 535                 540

Leu Lys Ser Gly Thr Cys Pro Phe Ser Phe Ser Lys Leu Asn Asn Phe
545                 550                 555                 560

Gln Lys Phe Lys Thr Ile Cys Phe Ser Thr Val Glu Val Pro Gly Ser
                565                 570                 575

Cys Asn Phe Pro Leu Glu Ala Thr Trp His Tyr Thr Ser Tyr Thr Ile
            580                 585                 590

Val Gly Ala Leu Tyr Val Thr Trp Ser Glu Gly Asn Ser Ile Thr Gly
        595                 600                 605

Val Pro Tyr Pro Val Ser Gly Ile Arg Glu Phe Ser Asn Leu Val Leu
    610                 615                 620

Asn Asn Cys Thr Lys Tyr Asn Ile Tyr Asp Tyr Val Gly Thr Gly Ile
625                 630                 635                 640

Ile Arg Ser Ser Asn Gln Ser Leu Ala Gly Gly Ile Thr Tyr Val Ser
                645                 650                 655

Asn Ser Gly Asn Leu Leu Gly Phe Lys Asn Val Ser Thr Gly Asn Ile
            660                 665                 670

Phe Ile Val Thr Pro Cys Asn Gln Pro Asp Gln Val Ala Val Tyr Gln
        675                 680                 685

Gln Ser Ile Ile Gly Ala Met Thr Ala Val Asn Glu Ser Arg Tyr Gly
    690                 695                 700

Leu Gln Asn Leu Leu Gln Leu Pro Asn Phe Tyr Tyr Val Ser Asn Gly
705                 710                 715                 720
```

```
Gly Asn Asn Cys Thr Thr Ala Val Met Thr Tyr Ser Asn Phe Gly Ile
                725                 730                 735

Cys Ala Asp Gly Ser Leu Ile Pro Val Arg Pro Arg Asn Ser Ser Asp
            740                 745                 750

Asn Gly Ile Ser Ala Ile Ile Thr Ala Asn Leu Ser Ile Pro Ser Asn
        755                 760                 765

Trp Thr Thr Ser Val Gln Val Glu Tyr Leu Gln Ile Thr Ser Thr Pro
    770                 775                 780

Ile Val Val Asp Cys Ala Thr Tyr Val Cys Asn Gly Asn Pro Arg Cys
785                 790                 795                 800

Lys Asn Leu Leu Lys Gln Tyr Thr Ser Ala Cys Lys Thr Ile Glu Asp
                805                 810                 815

Ala Leu Arg Leu Ser Ala His Leu Glu Thr Asn Asp Val Ser Ser Met
            820                 825                 830

Leu Thr Phe Asp Ser Asn Ala Phe Ser Leu Ala Asn Val Thr Ser Phe
        835                 840                 845

Gly Asp Tyr Asn Leu Ser Ser Val Leu Pro Gln Arg Asn Ile Arg Ser
    850                 855                 860

Ser Arg Ile Ala Gly Arg Ser Ala Leu Glu Asp Leu Leu Phe Ser Lys
865                 870                 875                 880

Val Val Thr Ser Gly Leu Gly Thr Val Asp Val Asp Tyr Lys Ser Cys
                885                 890                 895

Thr Lys Gly Leu Ser Ile Ala Asp Leu Ala Cys Ala Gln Tyr Tyr Asn
            900                 905                 910

Gly Ile Met Val Leu Pro Gly Val Ala Asp Ala Glu Arg Met Ala Met
        915                 920                 925

Tyr Thr Gly Ser Leu Ile Gly Gly Met Val Leu Gly Gly Leu Thr Ser
    930                 935                 940
```

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Ala Ala Ala Ile Pro Phe Ser Leu Ala Leu Gln Ala Arg Leu Asn Tyr
945                 950                 955                 960

Val Ala Leu Gln Thr Asp Val Leu Gln Glu Asn Gln Lys Ile Leu Ala
                965                 970                 975

Ala Ser Phe Asn Lys Ala Ile Asn Asn Ile Val Ala Ser Phe Ser Ser
            980                 985                 990

Val Asn Asp Ala Ile Thr Gln Thr  Ala Glu Ala Ile His  Thr Val Thr
        995                 1000                1005

Ile Ala  Leu Asn Lys Ile Gln  Asp Val Val Asn Gln  Gln Gly Ser
    1010                1015                1020

Ala Leu  Asn His Leu Thr Ser  Gln Leu Arg His Asn  Phe Gln Ala
    1025                1030                1035

Ile Ser  Asn Ser Ile Gln Ala  Ile Tyr Asp Arg Leu  Asp Ser Ile
    1040                1045                1050

Gln Ala  Asp Gln Gln Val Asp  Arg Leu Ile Thr Gly  Arg Leu Ala
    1055                1060                1065

Ala Leu  Asn Ala Phe Val Ser  Gln Val Leu Asn Lys  Tyr Thr Glu
    1070                1075                1080

Val Arg  Gly Ser Arg Arg Leu  Ala Gln Gln Lys Ile  Asn Glu Cys
    1085                1090                1095

Val Lys  Ser Gln Ser Asn Arg  Tyr Gly Phe Cys Gly  Asn Gly Thr
    1100                1105                1110

His Ile  Phe Ser Ile Val Asn  Ser Ala Pro Asp Gly  Leu Leu Phe
    1115                1120                1125

Leu His  Thr Val Leu Leu Pro  Thr Asp Tyr Lys Asn  Val Lys Ala
    1130                1135                1140

Trp Ser  Gly Ile Cys Val Asp  Gly Ile Tyr Gly Tyr  Val Leu Arg
    1145                1150                1155

Gln Pro  Asn Leu Val Leu Tyr  Ser Asp Asn Gly Val  Phe Arg Val
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1160 1165 1170

Thr Ser Arg Val Met Phe Gln Pro Arg Leu Pro Val Leu Ser Asp
1175 1180 1185

Phe Val Gln Ile Tyr Asn Cys Asn Val Thr Phe Val Asn Ile Ser
1190 1195 1200

Arg Val Glu Leu His Thr Val Ile Pro Asp Tyr Val Asp Val Asn
1205 1210 1215

Lys Thr Leu Gln Glu Phe Ala Gln Asn Leu Pro Lys Tyr Val Lys
1220 1225 1230

Pro Asn Phe Asp Leu Thr Pro Phe Asn Leu Thr Tyr Leu Asn Leu
1235 1240 1245

Ser Ser Glu Leu Lys Gln Leu Glu Ala Lys Thr Ala Ser Leu Phe
1250 1255 1260

Gln Thr Thr Val Glu Leu Gln Gly Leu Ile Asp Gln Ile Asn Ser
1265 1270 1275

Thr Tyr Val Asp Leu Lys Leu Leu Asn Arg Phe Glu Asn Tyr Ile
1280 1285 1290

Lys Trp Pro Trp Trp Val Trp Leu Ile Ile Ser Val Val Phe Val
1295 1300 1305

Val Leu Leu Ser Leu Leu Val Phe Cys Cys Leu Ser Thr Gly Cys
1310 1315 1320

Cys Gly Cys Cys Asn Cys Leu Thr Ser Ser Met Arg Gly Cys Cys
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Asp Cys Gly Ser Thr Lys Leu Pro Tyr Tyr Glu Phe Glu Lys Val
1340 1345 1350

His Val Gln
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Leu Trp Leu Leu Val Met Ile Phe Phe Phe Val Leu Ala Met Thr Phe
20 25 30

Ile Lys Leu Ile Gln Leu Cys Phe Thr Cys His Tyr Phe Phe Ser Arg
35 40 45

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50 55 60

Met Gln Ile Ala Pro Val Pro Ala Glu Val Leu Asn Val
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<223> ORF-5 pfam01635, Corona_M, Coronavirus M matrix/glycoprotein

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Asn Trp Asn Phe Ser Trp Asn Leu Ile Leu Thr Leu Phe Ile Val Val
20 25 30

Leu Gln Tyr Gly His Tyr Lys Tyr Ser Arg Leu Leu Tyr Gly Leu Lys
35 40 45

Met Ser Val Leu Trp Cys Leu Trp Pro Leu Val Leu Ala Leu Ser Ile

```
    50                  55                  60

Phe Asp Cys Phe Val Asn Phe Asn Val Asp Trp Val Phe Phe Gly Phe
65                  70                  75                  80

Ser Ile Leu Met Ser Ile Ile Thr Leu Cys Leu Trp Val Met Tyr Phe
                85                  90                  95

Val Asn Ser Phe Arg Leu Trp Arg Arg Val Lys Thr Phe Trp Ala Phe
            100                 105                 110

Asn Pro Glu Thr Asn Ala Ile Ile Ser Leu Gln Val Tyr Gly His Asn
        115                 120                 125

Tyr Tyr Leu Pro Val Met Ala Ala Pro Thr Gly Val Thr Leu Thr Leu
    130                 135                 140

Leu Ser Gly Val Leu Leu Val Asp Gly His Lys Ile Ala Thr Arg Val
145                 150                 155                 160

Gln Val Gly Gln Leu Pro Lys Tyr Val Ile Val Ala Thr Pro Ser Thr
                165                 170                 175

Thr Ile Val Cys Asp Arg Val Gly Arg Ser Val Asn Glu Thr Ser Gln
            180                 185                 190

Thr Gly Trp Ala Phe Tyr Val Arg Ala Lys His Gly Asp Phe Ser Gly
        195                 200                 205

Val Ala Ser Gln Glu Gly Val Leu Ser Glu Arg Glu Lys Leu Leu His
    210                 215                 220

Leu Ile
225


<210>  67
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<212>  PRT
<213>  Human coronavirus

<220>
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<223>  ORF-6 Pfam 00937, Coronavirus nucleocapsid protein
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1               5                  10                  15

Pro Pro Pro Ser Phe Tyr Met Pro Leu Leu Val Ser Ser Asp Lys Ala
            20                  25                  30

Pro Tyr Arg Val Ile Pro Arg Asn Leu Val Pro Ile Gly Lys Gly Asn
        35                  40                  45

Lys Asp Glu Gln Ile Gly Tyr Trp Asn Val Gln Glu Arg Trp Arg Met
    50                  55                  60

Arg Arg Gly Gln Arg Val Asp Leu Pro Pro Lys Val His Phe Tyr Tyr
65                  70                  75                  80

Leu Gly Thr Gly Pro His Lys Asp Leu Lys Phe Arg Gln Arg Ser Asp
                85                  90                  95

Gly Val Val Trp Val Ala Lys Glu Gly Ala Lys Thr Val Asn Thr Ser
            100                 105                 110

Leu Gly Asn Arg Lys Arg Asn Gln Lys Pro Leu Glu Pro Lys Phe Ser
        115                 120                 125

Ile Ala Leu Pro Pro Glu Leu Ser Val Val Glu Phe Glu Asp Arg Ser
    130                 135                 140

Asn Asn Ser Ser Arg Ala Ser Ser Arg Ser Ser Thr Arg Asn Asn Ser
145                 150                 155                 160

Arg Asp Ser Ser Arg Ser Thr Ser Arg Gln Gln Ser Arg Thr Arg Ser
                165                 170                 175

Asp Ser Asn Gln Ser Ser Ser Asp Leu Val Ala Ala Val Thr Leu Ala
            180                 185                 190

Leu Lys Asn Leu Gly Phe Asp Asn Gln Ser Lys Ser Pro Ser Ser Ser
        195                 200                 205

Gly Thr Ser Thr Pro Lys Lys Pro Asn Lys Pro Leu Ser Gln Pro Arg
    210                 215                 220
```

```
Ala Asp Lys Pro Ser Gln Leu Lys Lys Pro Arg Trp Lys Arg Val Pro
225                 230                 235                 240

Thr Arg Glu Glu Asn Val Ile Gln Cys Phe Gly Pro Arg Asp Phe Asn
                245                 250                 255

His Asn Met Gly Asp Ser Asp Leu Val Gln Asn Gly Val Asp Ala Lys
            260                 265                 270

Gly Phe Pro Gln Leu Ala Glu Leu Ile Pro Asn Gln Ala Ala Leu Phe
        275                 280                 285

Phe Asp Ser Glu Val Ser Thr Asp Glu Val Gly Asp Asn Val Gln Ile
    290                 295                 300

Thr Tyr Thr Tyr Lys Met Leu Val Ala Lys Asp Asn Lys Asn Leu Pro
305                 310                 315                 320

Lys Phe Ile Glu Gln Ile Ser Ala Phe Thr Lys Pro Ser Ser Ile Lys
                325                 330                 335

Glu Met Gln Ser Gln Ser Ser His Val Ala Gln Asn Thr Val Leu Asn
            340                 345                 350

Ala Ser Ile Pro Glu Ser Lys Pro Leu Ala Asp Asp Asp Ser Ala Ile
        355                 360                 365

Ile Glu Ile Val Asn Glu Val Leu His
    370                 375
```

Claims

1. An isolated and/or recombinant nucleic acid comprising a sequence as depicted in figure 19 and/or table 3, or a functional part, derivative and/or analogue thereof.

2. An isolated and/or recombinant nucleic acid that is at least 70% homologous to a nucleic acid according to claim 1.

3. An isolated and/or recombinant nucleic acid comprising that is at least 95% homologous to a nucleic acid according to claim 1.

4. An isolated and/or recombinant nucleic acid comprising a stretch of 100 consecutive nucleotides of a nucleic acid according to any one of claims 1-3.

5. An isolated and/or recombinant proteinaceous molecule comprising a sequence as depicted in figure 20, 21 ,22, 23 or table 3, or a functional part, derivative and/or analogue thereof.

6. An isolated and/or recombinant proteinaceous molecule that is at least 70% homologues to a proteinaceous molecule according to claim 5.

7. An isolated and/or recombinant proteinaceous molecule that is at least 95% homologues to a proteinaceous molecule according to claim 5.

8. An isolated and/or recombinant proteinaceous molecule comprising a stretch of at least 30 consecutive amino acids of a proteinaceous molecule according to any one of claims 5-7.

9. A nucleic acid encoding a proteinaceous molecule according to any one of claims 5-8.

10. An isolated or recombinant virus comprising a nucleic acid sequence according to any one of claims 1-4, or 9, or a functional part, derivative and/or analogue thereof.

11. An isolated or recombinant virus comprising a proteinaceous molecule according to any one of claims 5-8, or a functional part, derivative and/or analogue thereof.

12. An isolated or recombinant virus or a functional part, derivative or analogue according to claim 10 or claim 11 capable of inducing a HCoV-NL63-related disease.

13. A vector comprising a nucleic acid according to any one of claims 1-4 or 9.

14. A primer and/or probe, capable of specifically hybridizing to a nucleic acid of a virus or functional part, derivative or analogue according to any one of claims 1-4 or 9.

15. A primer and/or probe according to claim 8 or 9, which is capable of hybridizing to said nucleic acid under stringent conditions.

16. A primer and/or probe according to claim 14 or claim 15, comprising a sequence as depicted in table 3, table 7, table 10 and figures 16 to 18.

17. An isolated binding molecule capable of specifically binding a proteinaceous molecule according to any one of claims 5-8, and/or an isolated or recombinant virus according to any one of claims 10-12.

18. An isolated binding molecule capable of specifically binding a nucleic acid sequence of a virus or functional part, derivative or analogue according to any one of claims 1-4 or 9.

19. An isolated binding molecule capable of specifically binding at least part of a nucleic acid sequence as depicted in table 3.

20. An isolated binding molecule according to any one of claims 17-19 which is a proteinaceous molecule.

21. A method for producing a binding molecule according to any one of claims 17-20 comprising

- producing molecules capable of binding a virus or functional part, derivative or analogue according to any one of claims 10-12 or an isolated and/or recombinant proteinaceous molecule according to any one of claims 5-8, and
- selecting a proteinaceous binding molecule that is specific for said virus and or said proteinaceous molecule.

22. An isolated or recombinant virus which is immunoreactive with a molecule according to any one of claims 17-21.

23. Use of a virus or functional part, derivative and/or analogue according to any one of claims 10-12 for detecting a molecule capable of specifically binding said virus in a sample.

24. Use of an isolated and/or recombinant proteinaceous molecule according to any one of claims 5-8, for detecting a binding molecule capable of specifically binding a virus according to any one of claims 10-12, or functional part, derivative and/or analogue of said virus in a sample.

25. Use according to claim 24, wherein said binding molecule comprises a specific ligand and/or antibody for said virus.

26. Use of a primer and/or probe according to any one of claims 14-16, a binding molecule according to any one of claims 17-20, and/or a nucleic acid or functional part, derivative or analogue according to any one of claims 1-4, or 9 for detecting and/or identifying a HCoV-NL63 coronavirus in a sample.

27. Use according to claim 26 wherein said nucleic acid comprises a sequence as depicted in table 3.

28. A vaccine comprising a virus or functional part, derivative or analogue according to any one of claims 10-12.

29. A vaccine comprising a proteinaceous molecule according to any one of claims 5-8.

30. A vaccine comprising a binding molecule according to any one of claims 17-20.

31. A medicament comprising a binding molecule according to any one of claims 17-20.

32. Use of a virus or functional part, derivative or analogue according to any one of claims 10-12, for the preparation of a vaccine against a coronaviral genus related disease.

33. Use of a proteinaceous molecule according to any one of claims 5-8, for the preparation of a vaccine against a coronaviral genus related disease.

34. Use of a binding molecule according to any one of claims 17-20, for the preparation of a vaccine against a coronaviral genus related disease.

35. A method for detecting a coronavirus in a sample **characterized in that** a binding molecule according to any one of claims 17-20, or a primer and/or probe according to any one of claims 14-17 is used.

36. A method for detecting a binding molecule for a coronavirus **characterized in that** a virus according to any one of claims 10-12 or proteinaceous molecule according to any one of claims 5-8 is used.

37. A method according to claim 35 or claim 36 for diagnosis of a coronaviral genus related disease.

38. Use according to claim 37 wherein said coronaviral genus related disease comprises an HCoV-NL63 coronavirus related disease.

39. A method for detecting a virus or functional part, derivative or analogue according to any one of claims 10-12 in a sample, comprising hybridizing and/or amplifying a nucleic acid of said virus or functional part, derivative or analogue with a primer and/or probe according to any one of claims 14-16 and detecting hybridized and/or amplified product.

40. A diagnostic kit comprising a virus or functional part, derivative or analogue according to any one of claims 10-12, a binding molecule according to any one of claims 17-20, and/or a primer/probe according to any one of claims 14-16.

41. A method for treating an individual suffering from, or at risk of suffering from, an HCoV-NL63 related disease, comprising administering to said individual a vaccine or medicament according to any one of claims 28-31.

42. A method for determining whether an individual suffers from an HCoV-NL63 related disease, comprising obtaining a sample from said individual and detecting a HCoV-NL63 virus according to any one of claims 10-12 or functional part, derivative or analogue thereof in said sample.

43. A cell comprising an isolated and/or recombinant virus according to any one of claims 10-12, or a functional part, derivative and/or analogue thereof.

44. A cell according to claim 43, wherein said cell is a primate cell.

45. A cell according to claim 43 or 44, wherein said cell is a kidney cell.

46. A cell according to claim 44 or claim 45, wherein said cell is a monkey cell.

47. A proteinaceous molecule according to any one of claims 5-8, encoding a 3CL protease .

48. A method for determining whether a compound is capable of at least in part inhibiting a viral protease **characterized in that** said protease is a protease according to claim 47, or a functional part, derivative and/or analogue thereof.

49. A compound capable of at least in part inhibiting a viral protease according to claim 47.

50. A compound according to claim 49, wherein said compound comprises an amino acid sequence YNSTLQ or a functional part, derivative and/or analogue thereof.

51. A medicament for the treatment of an individual suffering from an coronavirus infection or an individual at risk of suffering there from comprising wherein said coronavirus comprises a nucleic acid sequence according to any one of claims 1-4, or 9, wherein said medicament comprises an amino acid sequence YNSTLQ or a functional part, derivative and/or analogue thereof..

52. Use of any of the hexapeptides located N-terminally of the putative $3Cl^{pro}$ cleavage sites for the preparation of a medicament for the treatment of an individual suffering or at risk of suffering from a coronavirus infection wherein said coronavirus comprises a sequence according to any one of claims 1-4, or 9.

53. A gene delivery vehicle comprising a sequence according to any one of claims 1-4 or 9.

54. A gene delivery vehicle according to claim 53, wherein said vector is based on a nucleic acid according to any one of claims 1-4, or 9.

55. An attenuated virus according to any one of claims 10-12.

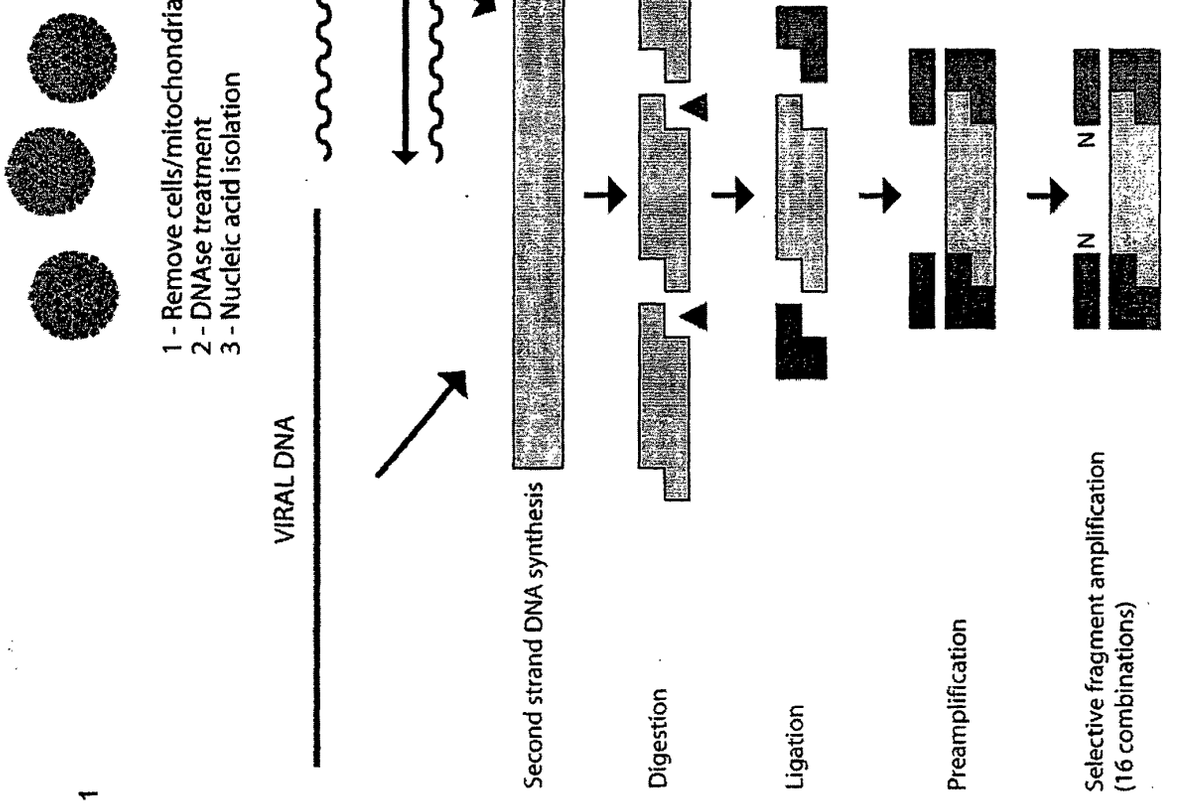
56. A polycistronic messenger RNA comprising ribosome slippery site that comprises a sequence that in the nucleic acid depicted in figure 19 is in position 12433 to 12439.

57. A vaccine according to claim 29, comprising at least an immunogenic part of the Spike protein having a sequence as depicted in figure 22.

58. A vaccine according to claim 57, wherein said part comprises a sequence from 20472 to 21009 of figure 19, or a functional part, derivative and/or analogue thereof.

59. A chimearic coronavirus comprising at least 1000 nucleotides of a sequence as depicted in figure 19 and at least 1000 nucleotides of another coronavirus wherein said latter 1000 nucleotides comprise a sequence that is more than 5% sequence divergent with a sequence as depicted in figure 19.

Figure 1


1 - Remove cells/mitochondria
2 - DNAse treatment
3 - Nucleic acid isolation
VIRAL DNA
VIRAL RNA
cDNA synthesis
Second strand DNA synthesis
Digestion
Ligation
Preamplification
Selective fragment amplification
(16 combinations)
Restriction Enzymes
Anchors
Primers
Selective primers
N
N
N
N

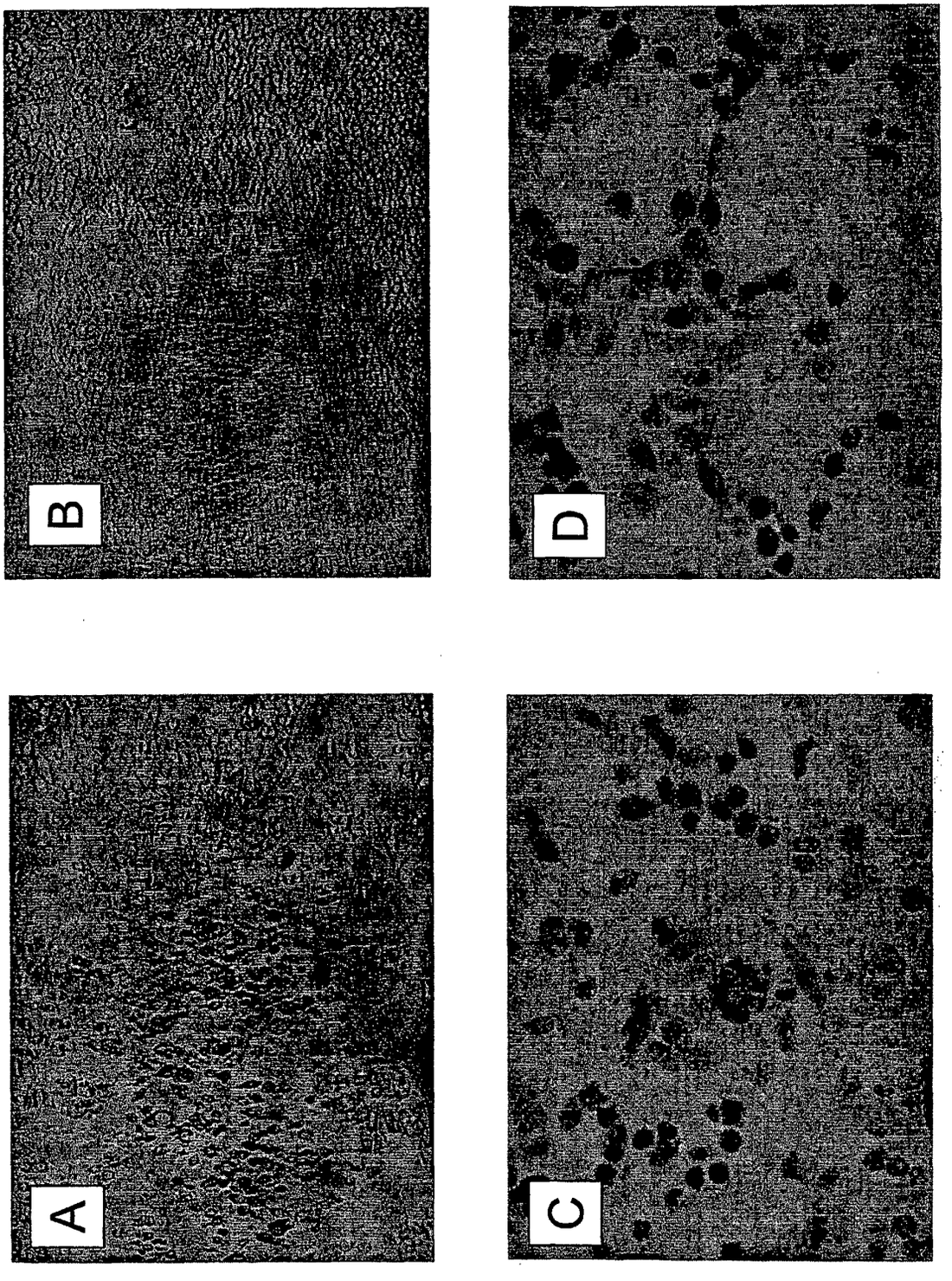


Figure 2

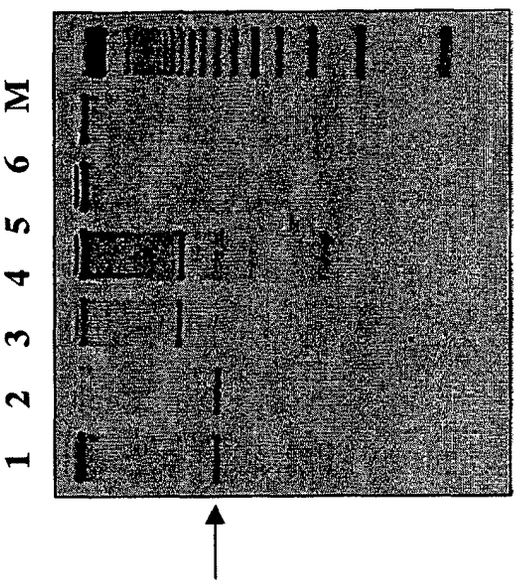
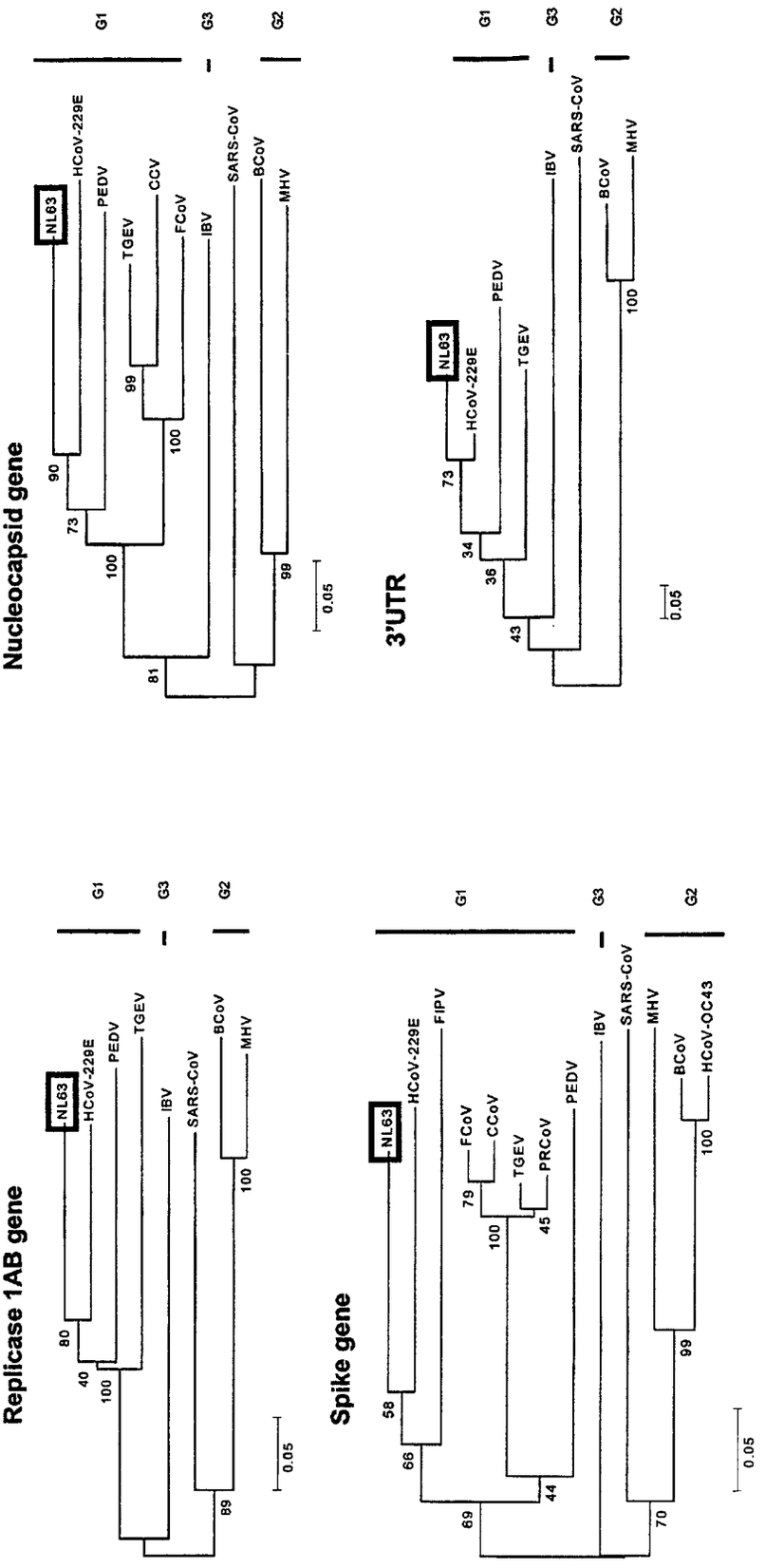


Figure 3

Figure 4


Nucleocapsid gene
G1
G3
G2
NL63
HCoV-229E
PEDV
TGEV
CCV
FCoV
IBV
SARS-CoV
BCoV
MHV
90
73
100
99
100
81
99
0.05
3'UTR
G1
G3
G2
NL63
HCoV-229E
PEDV
TGEV
IBV
SARS-CoV
BCoV
MHV
73
34
36
43
100
0.05
Replicase 1AB gene
G1
G3
G2
NL63
HCoV-229E
PEDV
TGEV
IBV
SARS-CoV
BCoV
MHV
80
40
100
89
100
0.05
Spike gene
G1
G3
G2
NL63
HCoV-229E
FIPV
FCoV
CCoV
TGEV
PRCoV
PEDV
IBV
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69
70
99
100
0.05

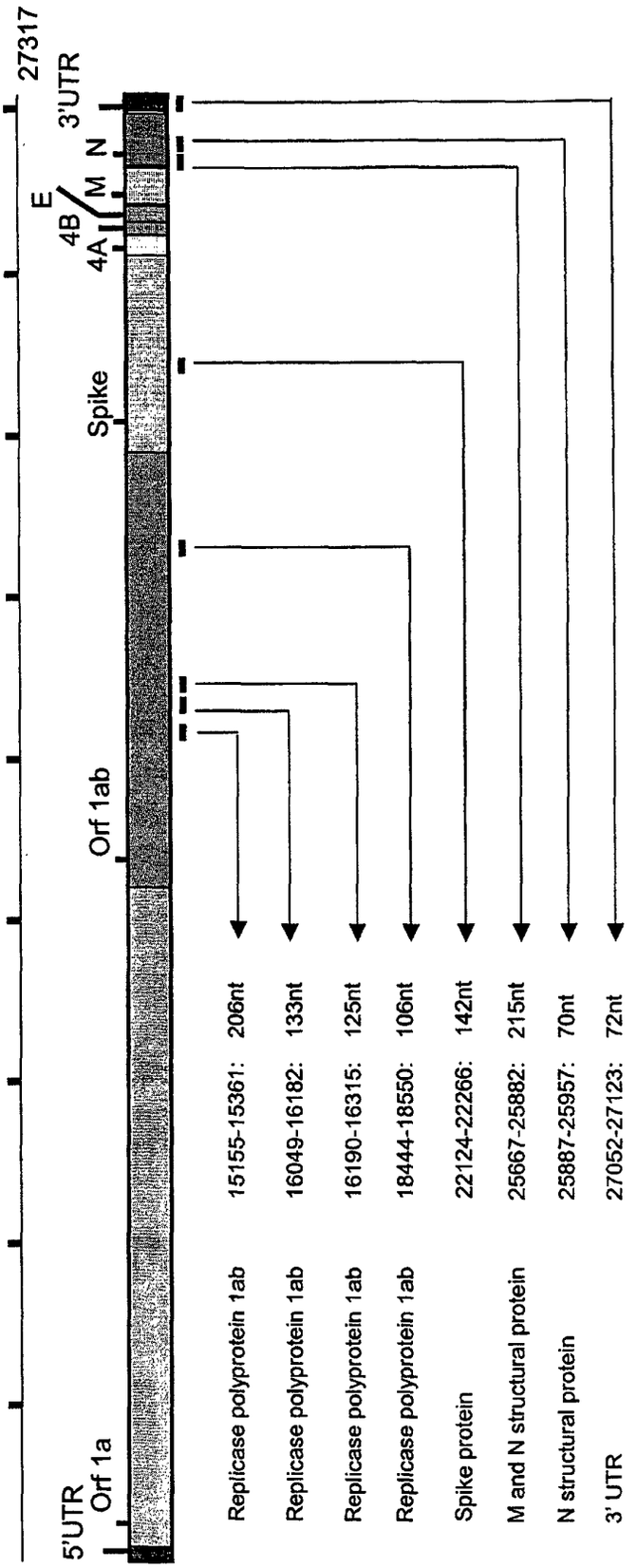
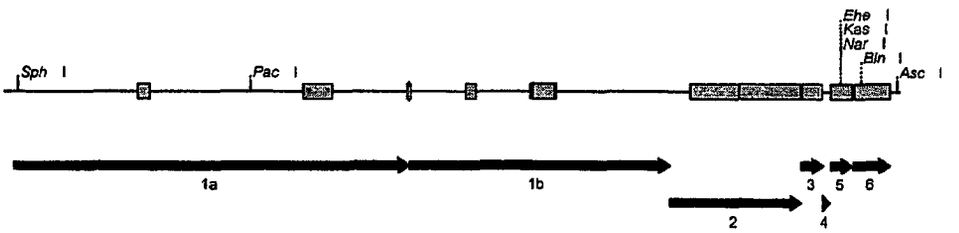


Figure 5, a schematic representation of the coronavirus genome organization (with HCoV-229E accession number AF304460 as an example). Red lines indicate the genome location of the sequence fragments of the new coronavirus HCoV-SLA163. UTR denotes unranslated region, orf: open reading frame, E: envelope protein, M: membrane protein, N: nucleocapsid protein.

Figure 6



HCoV-NL63

Figure 7

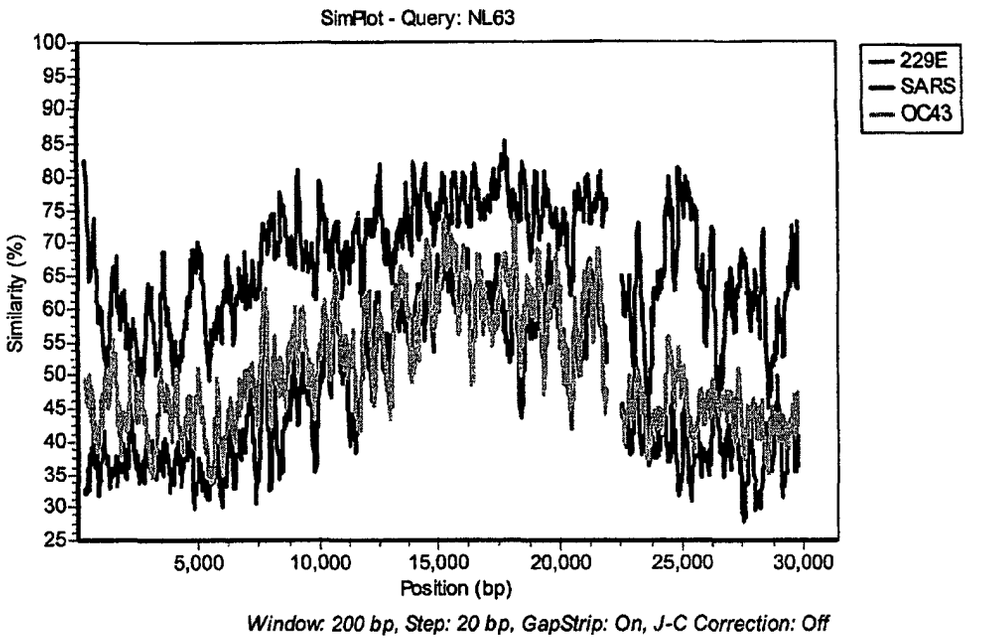
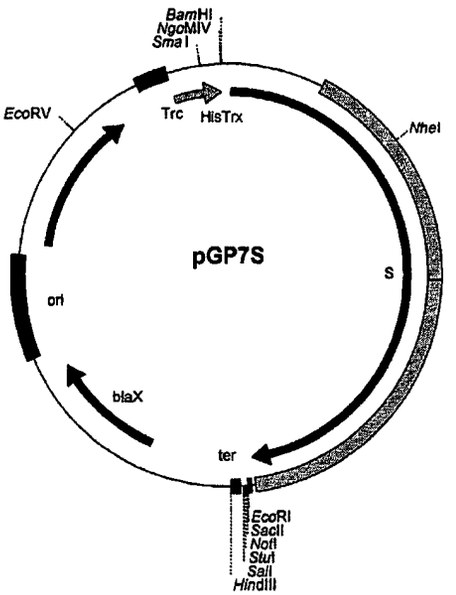

SimPlot - Query: NL63
229E
SARS
OC43
Similarity (%)
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
5,000
10,000
15,000
20,000
25,000
30,000
Position (bp)
Window: 200 bp, Step: 20 bp, GapStrip: On, J-C Correction: Off

Figure 8


BamHI
NgoMIV
Sma I
EcoRV
Trc
HisTrx
NheI
pGP7S
S
ori
blaX
ter
EcoRI
SacII
NotI
StuI
SalI
HindIII

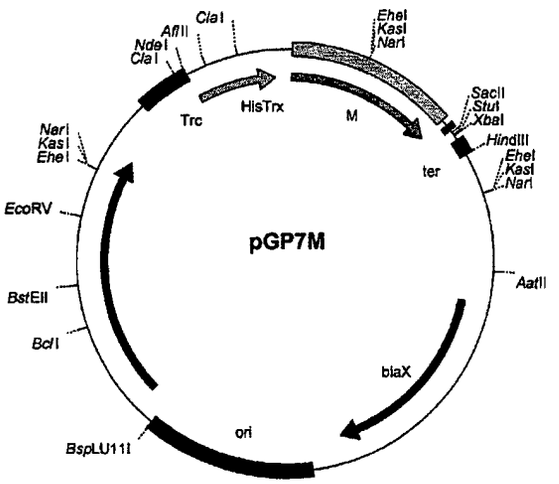
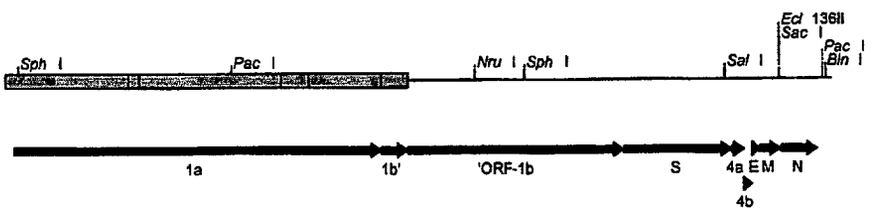

ClaI
AflII
NdeI
ClaI
EheI
KasI
NarI
SacII
StuI
XbaI
Trc
HisTrx
M
HindIII
EheI
KasI
NarI
ter
NarI
KasI
EheI
EcoRV
pGP7M
AatII
BstEII
BclI
blaX
ori
BspLU11I

Figure 9

TCATCCTAATTGTTGTGACTGTTATGATGATATGTGTGTTATACATTGTTCAAATTTTAACA
CACTCTT

Figure 10

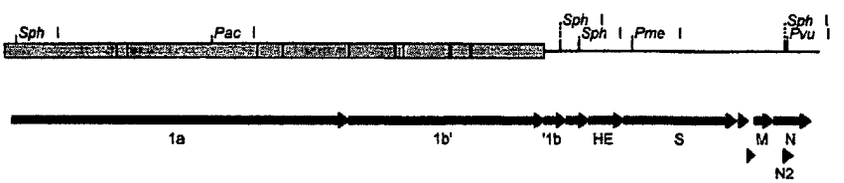


Chimera NL63/229E

Figure 11

CAACGTATGTGTTTGGAACCTTGTAATTTATATAATTATGGGAAGCCAGTTACTTTGCCT

Figure 12



Chimera NL63/OC43

Figure 13

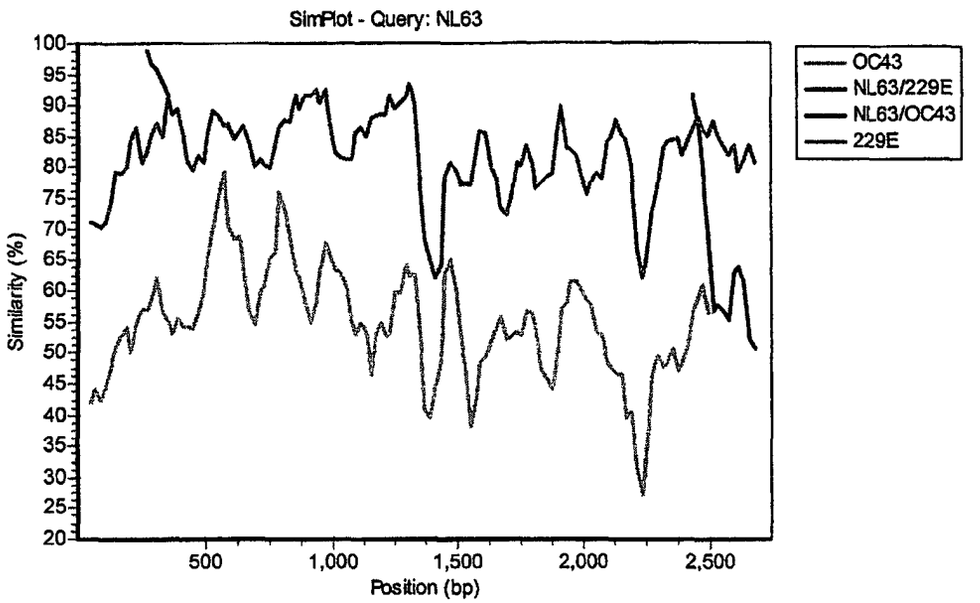
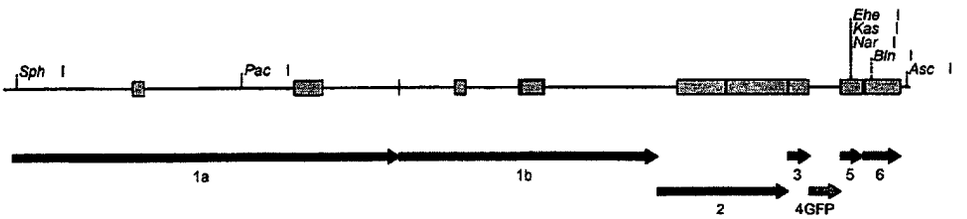
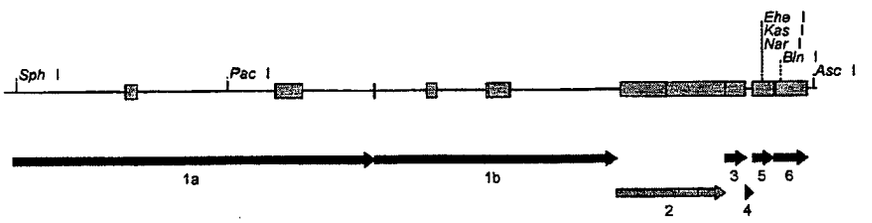

SimPlot - Query: NL63
Similarity (%)
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
500
1,000
1,500
2,000
2,500
Position (bp)
OC43
NL63/229E
NL63/OC43
229E
Window: 100 bp, Step: 20 bp, GapStrip: On, J-C Correction: Off

Figure 14



HCoV-NL63/4GFP

Figure 15



NL63_D20520-21011

Figure 16

Sequence variation in HCoV-NL63 from additional patient samples

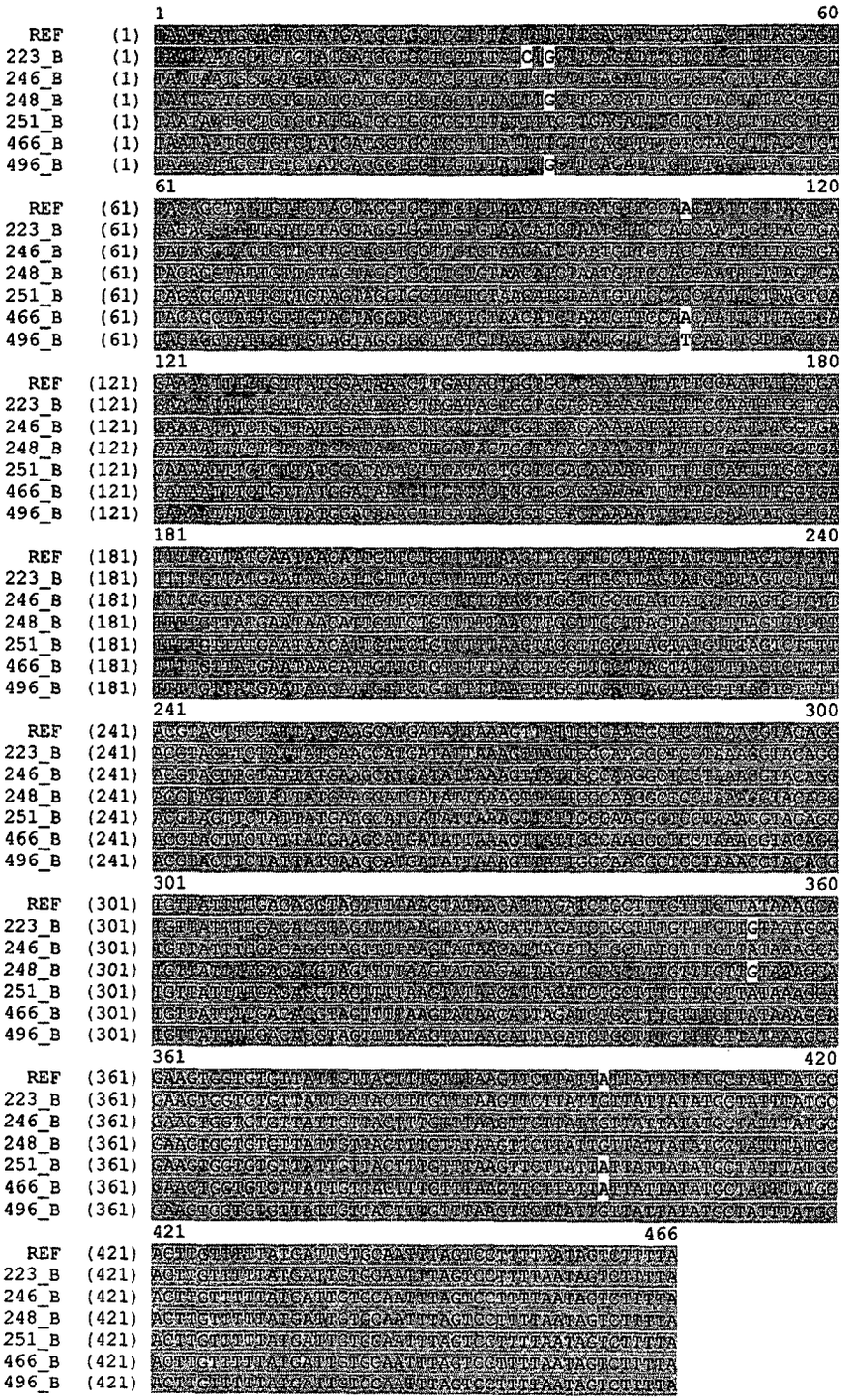


Figure 17

HCoV-NL63 Nucleocapsid (N) specific PCR primers

Oligo	Sequence	T_m	Amplicon
NL63NF1	GCTAGTGTAAATTGGGCCGATG	55.3	708
NL63NR1	CTTCCAACGAGGTTTCTTCAACTG	56.0	
NL63NF2	TCCTCCTCCTTCATTTTACATGCC	57.4	372
NL63NR2	AACTCAACAACAGAGAGCTCTGGAG	55.1	

Figure 18

Generic Coronavirus detection primers

Oligo	Sequence	T_m	Amplicon
COR1F	ATGGGWTGGGAYTATCCIAARTGTGA	60.6	603
COR1R	GYTGKGARCARAAYTCRTGWGGTCC	60.7	
COR2F	TATKTTAARCCWGGTGGIAC	46.1	362
COR2R	CATRAANACRYYATTYTGRTAATA	46.7	

Figure 19

Nucleotide sequence HcoV_NL63

CTTAAAGAATTTTTCTATCTATAGATAGAGAATTTTCTTATTTAGACTTTGTGTCTACTC
TTCTCAACTAAACGAAATTTTTCTAGTGCTGTCATTTGTTATGGCAGTCCTAGTGTAATT
GAAATTTCGTCAAGTTTGTAAACTGGTTAGGCAAGTGTTGTATTTTCTGTGTCTAAGCAC
TGGTGATTCTGTTCACTAGTGCATACATTGATATTTAAGTGGTGTTCCGTCACTGCTTAT
TGTGGAAGCAACGTTCTGTCGTTGTGGAAACCAATAACTGCTAACCATGTTTTACAATCA
AGTGACACTTGCTGTTGCAAGTGATTCGGAAATTTCAGGTTTTGGTTTTGCCATTCCTTC
TGTAGCCGTTCGCACCTATAGCGAAGCCGCTGCACAAGGTTTTCAGGCATGCCGTTTTGT
TGCTTTTGGCTTACAGGATTGTGTAACCGGTATTAATGATGATGATTATGTCATTGCATT
GACTGGTACTAATCAGCTCTGTGCCAAAATTTTACCTTTTTCTGATAGACCCCTTAATTT
GCGAGGTTGGCTCATTTTTTCTAACAGCAATTATGTTCTTCAGGACTTTGATGTTGTTTT
TGGCCATGGTGCAGGAAGTGTGGTTTTTGTGGATAAGTACATGTGTGGTTTTGATGGTAA
ACCTGTGTTACCTAAAAACATGTGGGAATTTAGGGATTACTTTAATAATAATACTGATAG
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TTATGAACAGCAAAATGTTTTAGCCATTGAGAGCATTCATTACCTTGGTACTACAGGTCA
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CATCACCTCAACTAGTGCTGGTTGTGGTGTTAAATACTATGCTGGCTTAGTTGTTAAACA
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GTTTGTGGCATCATCTTCTTATGATGCACTCTTGCATAGAAATTCATTAGACCCTTTTTG
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TAATGGTGTATTGCCTGTTGCTTTTACAAAATTAGCTGGTGGTAAAATATCTTTTTCTGA
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Figure 19 (Cont.)

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Figure 19 (Cont)

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Figure 19 (Cont)

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AGCTGACCTTAAATTTGTTAAGTGGGAGTATGAGGGTGGTTGCAACACAATCGAGTTAGA
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TCGTCTACAAGCTGGTAAACAAACTGAATTGGCTGTTAATTCTGGACTTTTAACTGCTTG
TGCTTTTTCTGTTGATCCAGCAACTACTTACTTGGAAGCTGTTAAACATGGTGCAAAACC
TGTAAGTAATTGTATTAAGATGTTATCTAATGGTGCTGGTAATGGTCAAGCTATAACAAC
TAGTGTAGATGCTAACACCAATCAAGATTCTTATGGTGGAGCGTCTATTTGTTTGTATTG
TCGGGCCCACGTTCCTCACCCTAGTATGGATGGTTACTGTAAGTTTAAGGGTAAATGTGT
TCAGGTTCCTATTGGTTGTTTGGATCCTATTAGGTTTTGTTTAGAAAATAATGTGTGTAA
TGTTTGTGGTTGTTGGTTGGGACACGGGTGTGCTTGTGACCGTACAACTATTCAAAGTGT
TGACATTTCTTATTTAAACGAGCAAGGGGTTCTAGTGCAGCTCGACTAGAACCCTGCAAT
GGCACGGACATCGATAAGTGTGTTCGTGCTTTTGACATTTATAATAAAAATGTTTCATTC
TTGGGTAAGTGTTTGAAGATGAACTGTGTTCGTTTTAAAAATGCTGATCTTAAGGATGGT
TATTTTGTTATAAAGAGGTGTACTAAGTCGGTTATGGAACACGAGCAATCCATGTATAAC
CTACTTAACTTTTCTGGTGCTTTGGCTGAGCATGATTTCTTTACTTGGAAAGATGGCAGA
GTCATTTATGGTAATGTTAGTAGACATAATCTTACTAAATATACTATGATGGACTTGGTC
TATGCTATGCGTAACTTTGATGAACAAAATTGTGATGTTCTAAAAGAAGTATTAGTTTTA
ACTGGTTGTTGTGACAATTCTTATTTTGATAGTAAGGGTTGGTATGACCCAGTTGAAAAT
GAAGATATACATAGAGTTTATGCATCTCTTGGCAAAATTGTAGCTAGAGCTATGCTTAAA
TGCGTTGCTCTATGCGATGCGATGGTTGCTAAAGGTGTTGTTGGTGTTTTAACATTAGAT

Figure 19 (Cont)

AACCAAGATCTTAATGGTAACTTTTATGATTTTGGTGATTTTGTTGTTAGCTTACCTAAT
ATGGGTGTTCCCTGTTGTACATCATATTATTCTTATATGATGCCTATTATGGGTTTAACT
AATTGTTTAGCTAGTGAGTGTTTTGTCAAGAGTGATATTTTTGGTAGTGATTTTAAAACT
TTTGATTTGCTTAAGTATGATTTCACTGAACATAAAGAAAATTTATTCAATAAGTACTTT
AAGCATTGGAGTTTTGATTATCATCCTAATTGTTGTGACTGTTATGATGATATGTGTGTT
ATACATTGTGCTAATTTTAATACACTATTTGCCACAACTATACCAGGTACTGCTTTTGGT
CCACTATGTCGTAAAGTTTTTATAGATGGTGTTCCACTTGTTACAACTGCTGGTTATCAT
TTTAAGCAATTAGGTTTGGTTTGGAATAAAGATGTTAACACACACTCAGTTAGGTTGACA
ATTACTGAACTTTTGCAATTTGTCACCGACCCTTCCTTGATAATAGCTTCTTCCCCAGCA
CTCGTTGATCAACGCACTATTTGTTTTTCTGTTGCAGCATTGAGTACTGGTTTGACAAAT
CAAGTTGTTAAGCCAGGTCATTTTAATGAAGAGTTTTATAACTTTCTTCGTTTAAGAGGT
TTCTTTGATGAAGGTTCTGAACTTACATTAAAACATTTCTTCTTCGCACAGAATGGTGAT
GCTGCTGTTAAAGATTTTGACTTTTACCGTTATAATAAGCCTACCATTTTAGATATTTGT
CAAGCTAGAGTTACATATAAGATAGTCTCTCGTTATTTTGACATTTATGAAGGTGGCTGT
ATTAAGGCATGTGAAGTTGTTGTAACAAATCTTAATAAGAGTGCTGGTTGGCCATTAAAT
AAGTTTGGTAAAGCTAGTTTGTATTATGAATCTATATCTTATGAAGAACAGGATGCTTTG
TTTGCTTTGACAAAGCGTAATGTCCTCCCTACTATGACACAGCTGAATCTTAAGTATGCT
ATTAGTGGTAAAGAACGTGCTAGAACTGTTGGTGGTGTTTCTCTGTTGTCTACAATGACC
ACAAGACAATACCATCAAAAACATCTTAAATCCATTGTTAATACACGCAATGCCACTGTT
GTTATTGGTACTACCAAATTTTATGGTGGTTGGAATAATATGTTGCGTACTTTAATTGAT
GGTGTTGAAAACCCTATGCTTATGGGTTGGGATTATCCCAAATGTGATAGAGCTTTGCCT
AACATGATACGTATGATTTCAGCCATGGTGTTGGGCTCTAAGCATGTTAATTGTTGTACT
GCAACAGATAGGTTTTATAGGCTTGGTAATGAGTTGGCACAAGTTTTAACAGAAGTTGTT
TATTCTAATGGTGGTTTTTATTTTAAGCCAGGTGGTACGACTTCTGGTGACGCTAGTACA
GCTTATGCTAATTCTATTTTTAACATTTTTCAAGCCGTGAGTTCTAACATTAACAGGTTG
CTTAGTGTCCCATCAGATTCATGTAATAATGTTAATGTTAGGGATCTACAACGACGTCTG
TATGATAATTGTTATAGGTTAACTAGTGTTGAAGAGTCATTCATTGAAGATTATTATGGT
TATCTTAGGAAACATTTTTCAATGATGATTCTCTCTGATGACGGTGTTGTCTGTTATAAC
AAGGATTATGCTGAGTTAGGTTATATAGCAGACATTAGTGCTTTTAAAGCCACTTTGTAT
TACCAGAATAATGTCTTTATGAGTACTTCTAAATGTTGGGTTGAAGAAGATTTAACTAAG
GGACCACATGAGTTTTGTTCCCAGCATACTATGCAAATAGTTGACAAAGATGGTACCTAT
TATTTGCCTTACCCAGATCCTAGTAGGATCTTGTCAGCTGGTGTTTTTGTTGATGATGTT
GTTAAGACAGATGCTGTTGTTTTGTTAGAACGTTATGTGTCTTTAGCTATTGATGCATAC
CCTCTTTCAAAACACCCTAATTCCGAATATCGTAAGGTTTTTTACGTATTACTTGATTGG
GTTAAGCATCTTAACAAAAATTTGAATGAGGGTGTTCTTGAATCTTTTTCTGTTACACTT
CTTGATAATCAAGAAGATAAGTTTTGGTGTGAAGATTTTTATGCTAGTATGTATGAAAAT
TCTACAATATTGCAAGCTGCTGGTTTATGTGTTGTTTGTGGTTCACAAACTGTACTTCGT
TGTGGTGATTGTCTGCGTAAGCCTATGTTGTGCACTAAATGCGCATATGATCATGTATTT
GGTACCGACCACAAGTTTATTTTGGCTATAACACCGTATGTATGTAATGCATCAGGTTGT
GGTGTTAGTGATGTCAAAAAATTGTATCTTGGTGGTTTGAATTACTATTGTACAAATCAT
AAACCACAGTTGTCTTTTCCATTATGTTCAGCTGGTAATATATTTGGTTTATATAAAAAT
TCAGCAACTGGTTCCTTAGATGTTGAAGTTTTTAATAGGCTTGCAACGTCTGATTGGACT
GATGTTAGGGACTATAAACTTGCTAATGATGTTAAAGATACACTTAGACTCTTTGCGGCT
GAAACTATTAAAGCTAAAGAAGAGAGTGTTAAGTCTTCTTATGCTTTTGCAACTCTTAAA
GAGGTTGTTGGACCTAAAGAATTGCTTCTTAGTTGGGAAAGTGGTAAAGTTAAACCACCT
TTGAATCGTAATTCTGTTTTCACTTGTTTTCAAATAAGTAAGGACTCAAAATTCCAAATA
GGTGAGTTCATCTTTGAGAAGGTTGAATATGGTTCTGATACTGTTACGTATAAGTCTACT
GTAACTACTAAGTTAGTTCCTGGTATGATTTTTGTCTTAACATCTCACAATGTCCAACCT
TTACGTGCACCAACTATTGCAAACCAAGAGAAGTATTCTAGCATTTATAAATTGCACCCT
GCTTTTAATGTCAGTGATGCATATGCTAATTTGGTTCCATATTACCAACTTATTGGTAAA
CAAAAGATAACTACAATACAGGGTCCTCCTGGTAGTGGTAAGTCACATTGTTCCATTGGA
CTTGGATTGTACTACCCAGGTGCGCGTATTGTTTTTGTTGCTTGTGCCCATGCTGCTGTT
GATTCCTTATGTGCAAAAGCTATGACTGTTTATAGCATTGATAAGTGTACTAGGATTATA
CCTGCAAGAGCTCGGGTTGAGTGTTATAGTGGCTTTAAACCAAATAACACTAGTGCACAA
TACATATTTAGCACTGTTAACGCATTACCTGAGTGTAATGCTGATATCGTTGTTGTAGAT

Figure 19 (Cont)

GAAGTTTCAATGTGTACAAATTATGACCTTTCTGTTATTAACCAGCGTTTATCATATAAA
CATATTGTTTATGTTGGTGATCCACAACAACTTCCTGCACCTAGAGTAATGATTACTAAA
GGTGTTATGGAGCCTGTTGATTATAACGTTGTTACTCAACGTATGTGTGCTATAGGCCCT
GATGTTTTTCTTCATAAATGTTATAGATGTCCTGCTGAAATAGTAATACAGTTTCTGAAC
TTGTTTATGAGAACAAGTTTGTCCCTGTTAAACCTGCTAGTAAACAGTGTTTTAAAGTCT
TTTTTAAGGGTAATGTACAAGGTTGACAATGGTTCTAGTATTAACAGAAAGCAGCTTGAA
ATAGTTAAGCTGTTTTTAGTTAAAAATCCAAGTTGGAGTAAGGCTGTGTTTATTTCTCCT
TATAATAGTCAGAATTATGTTGCTAGTAGATTTTTAGGACTTCAAATTCAAACTGTTGAT
TCTTCTCAAGGTAGTGAGTATGATTATGTAATCTATGCACAAACTTCTGACACTGCACAT
GCTTGCAATGTAAACCGTTTTAATGTTGCTATAACACGTGCTAAGAAGGGTATATTTTGT
GTAATGTGTGATAAAACTTTGTTTGATTCACTTAAGTTTTTTGAGATTAAACATGCAGAT
TTACACTCTAGCCAGGTTTGTGGCTTGTTTAAAAATTGTACACGCACTCCTCTTAATTTA
CCACCAACTCATGCACACACTTTCTTGTCGTTGTCAGATCAGTTTAAGACTACAGGTGAT
TTAGCTGTTCAAATAGGTTCAAATAACGTTTGTACTTATGAACATGTTATATCATTTATG
GGTTTTAGGTTTGATATTAGTATTCCTGGTAGTCATAGTTTGTTTTGTACACGTGACTTT
GCTATTCGTAATGTGCGTGGTTGGTTGGGTATGGATGTTGAAAGTGCTCATGTTTGTGGC
GATAACATAGGTACTAATGTTCCTTTACAGGTTGGTTTTTCAAATGGTGTTAATTTTGTT
GTGCAAACTGAAGGTTGTGTGTCTACCAATTTTGGTGATGTTATTAAACCTGTTTGTGCA
AAATCTCCACCAGGTGAACAATTTAGACACCTTATTCCTCTTTTACGTAAAGGACAACCT
TGGTTAATTGTTCGTAGACGCATTGTGCAAATGATATCTGATTATTTGTCCAATTTGTCT
GACATTCTTGTCTTTGTTTTGTGGGCAGGTAGTTTGGAATTAACTACAATGCGTTACTTT
GTAAAAATAGGGCCAATTAAATATTGTTATTGTGGTAATTTTGCCACTTGTTATAATTCA
GTTAGTAATGAATATTGTTGTTTTAAACATGCATTGGGTTGTGATTATGTTTACAATCCG
TATGCTTTTGATATACAACAGTGGGGTTATGTTGGTTCCTTGAGCCAAAACCACCACACA
TTCTGTAACATTCATAGAAACGAGCATGATGCCTCTGGTGATGCTGTTATGACACGTTGT
TTGGCAGTACATGATTGTTTTGTCAAAAATGTTGATTGGACTGTAACGTACCCCTTTATT
GCAAATGAGAAATTTATCAATGGCTGTGGGCGTAATGTCCAGGGACATGTTGTTCGTGCA
GCCTTGAAATTGTATAAACCTAGTGTTATTCATGACATTGGTAATCCTAAAGGTGTACGT
TGTGCTGTTACTGATGCCAAATGGTACTGTTATGACAAGCAACCTGTTAATAGTAATGTC
AAGTTGTTGGATTATGATTATGCAACCCATGGTCAACTTGATGGTCTTTGTTTATTCTGG
AATTGTAATGTTGATATGTATCCAGAATTTTCAATTGTGTGTCGTTTTGACACACGTACT
CGTTCTGTTTTTAATTTAGAAGGTGTTAATGGTGGTTCTCTTTATGTTAACAAACATGCG
TTTCATACACCAGCATATGATAAACGTGCTTTTGTTAAATTAAAACCTATGCCCTTTTTT
TACTTTGATGACAGTGATTGTGATGTTGTGCAAGAACAAGTTAATTATGTACCCCTTCGC
GCTAGTAGTTGTGTTACTCGTTGTAATATAGGTGGTGCTGTTTGTTCAAAACATGCAAAT
TTGTATCAAAAATATGTTGAGGCATATAATACATTTACACAGGCAGGTTTTAACATTTGG
GTACCACATAGTTTTGATGTTTATAATTTGTGGCAAATTTTTATTGAAACTAATTTACAA
AGTCTTGAAAATATAGCATTTAATGTTGTAAAAAAAGGGTGTTTTACTGGTGTTGATGGT
GAGTTACCTGTTGCAGTTGTTAACGACAAAGTTTTTGTTCGCTATGGCGATGTTGACAAC
TTGGTTTTTACAAATAAAACAACATTGCCTACTAATGTTGCTTTTGAATTGTTTGCAAAA
CGAAAAATGGGTTTAACACCACCATTGTCTATTCTCAAAAATCTCGGTGTTGTTGCTACA
TATAAATTTGTTTTATGGGATTATGAAGCTGAAAGACCTTTTACCTCATATACTAAGAGT
GTATGTAAATACACTGATTTTAATGAGGATGTTTGTGTTTGTTTTGACAATAGTATTCAG
GGTTCGTATGAGCGTTTTACGCTTACTACGAACGCTGTTTTATTTTCTACTGTTGTCATT
AAAAATTTAACACCTATAAAGTTGAATTTTGGTATGTTGAATGGTATGCCAGTTTCTTCT
ATTAAGGGTGATAAAGGTGTTGAAAAATTAGTTAATTGGTACATATATGTTCGTAAAAAT
GGTCAATTTCAAGATCACTATGATGGTTTTTACACTCAAGGTAGGAATTTATCAGACTTT
ACACCAAGAAGTGATATGGAGTATGATTTTCTTAACATGGATATGGGTGTTTTTATTAAT
AAATATGGTCTTGAGGATTTTAATTTTGAACATGTTGTATATGGTGATGTTTCAAAAACT
ACATTAGGAGGTCTTCATTTGTTGATATCACAGTTTAGGCTTAGTAAAATGGGTGTTTTG
AAAGCTGATGATTTTGTCACTGCTTCTGACACAACTTTGAGGTGCTGTACTGTTACTTAT
CTTAATGAACTTAGTTCAAAAGTTGTTTGTACTTATATGGATTTGTTGTTGGACGACTTT
GTTACTATACTAAAGAGTTTAGATCTTGGTGTAATATCTAAAGTTCATGAAGTTATTATA
GATAATAAACCTTATAGGTGGATGTTGTGGTGTAAAGATAACCACTTGTCCACTTTTTAT
CCACAGTTGCAGTCTGCTGAATGGAAGTGTGGTTATGCTATGCCACAAATTTATAAGCTT

Figure 19 (Cont)

CAACGTATGTGTTTGGAACCTTGTAATTTATATAATTATGGTGCTGGTATTAAGTTGCCT
AGTGGTATAATGTTAAATGTTGTTAAATACACTCAGCTTTGTCAATACCTAAATAGCACT
ACAATGTGCGTACCTCATAATATGCGTGTTTTGCACTATGGTGCTGGTTCTGACAAAGGT
GTGGCACCTGGTACAACTGTTTTAAAACGTTGGCTACCACCCGATGCAATAATCATTGAT
AATGATATCAATGATTATGTTAGTGATGCAGATTTTAGCATTACAGGTGATTGTGCTACT
GTTTATCTTGAAGATAAGTTTGACTTACTTATTTCTGATATGTATGATGGTAGAATTAAA
TTTTGTGATGGTGAAAATGTCTCTAAAGATGGGTTTTTTACTTATCTTAATGGTGTTATT
AGAGAAAAATTAGCTATTGGTGGTAGTGTTGCCATTAAGATTACAGAATATAGTTGGAAT
AAGTATCTTTATGAATTAATACAAAGATTTGCTTTTTGGACTTTGTTTTGCACGTCTGTT
AATACATCCTCTTCAGAAGCTTTTCTTATTGGTATTAATTATTTAGGTGACTTTATTCAA
GGTCCTTTTATAGCTGGTAACACTGTTCATGCTAATTATATATTTTGGCGTAATTCTACT
ATTATGTCTTTGTCATACAATTCAGTTTTAGATTTAAGTAAGTTTGAATGTAAACATAAA
GCCACTGTTGTTGTTACACTTAAAGATAGTGATGTAAATGATATGGTTTTGAGTTTGATT
AAGAGTGGTAGGTTGTTGTTACGCAATAATGGTCGTTTTGGTGGTTTTAGTAATCATTTA
GTCTCAACTAAATGAAACTTTTCTTGATTTTGCTTGTTTTGCCCCTGGCCTCTTGCTTTT
TCACATGTAATAGTAATGCTAATCTCTCTATGTTACAATTAGGTGTTCCTGACAATTCTT
CAACTATTGTTACGGGTTTATTGCCAACTCATTGGTTTTGTGCTAATCAGAGTACATCTG
TTTACTCAGCCAATGGTTTCTTTTATATTGATGTTGGTAATCACCGTAGTGCTTTTGCGC
TCCATACTGGTTATTATGATGCTAATCAGTATTATATTTATGTTACTAATGAAATAGGCT
TAAATGCTTCTGTTACTCTTAAGATTTGTAAGTTTAGTAGAAACACTACTTTTGATTTTT
TAAGTAATGCTTCTAGTTCTTTTGACTGTATAGTTAATTTGTTATTTACAGAACAGTTAG
GTGCGCCTTTGGGCATAACTATATCTGGTGAAACTGTGCGTCTGCATTTATATAATGTAA
CTCGTACTTTTTATGTGCCAGCAGCTTATAAACTTACTAAACTTAGTGTTAAATGTTACT
TTAACTATTCCTGTGTTTTTAGTGTTGTCAACGCCACCGTTACTGTGAATGTCACCACAC
ATAATGGCCGTGTAGTTAACTACACTGTTTGTGATGATTGTAATGGTTATACTGATAACA
TATTTTCTGTTCAACAGGATGGCCGCATTCCTAATGGTTTCCCTTTTAATAATTGGTTTT
TGTTAACTAATGGTTCCACACTAGTGGACGGGGTCTCTAGACTTTATCAACCACTCCGTT
TAACTTGTTTATGGCCTGTACCTGGTCTTAAATCTTCAACTGGTTTTGTTTATTTTAATG
CCACTGGTTCTGATGTTAATTGTAACGGCTATCAACATAATTCTGTTGTTGATGTTATGC
GTTACAATCTTAACTTCAGTGCTAATTCTTTGGACAATCTCAAGAGTGGTGTTATAGTTT
TTAAAACTTTACAGTACGATGTTTTGTTTTATTGTAGTAATTCTTCCTCAGGTGTTCTTG
ACACCACAATACCTTTTGGCCCGTCCTCTCAACCTTATTACTGTTTTATAAACAGCACTA
TCAACACTACTCATGTTAGCACTTTTGTGGGTATTTTACCACCCACTGTGCGTGAAATTG
TTGTTGCTAGAACTGGCCAGTTTTATATTAATGGTTTTAAGTATTTCGATTTGGGTTTCA
TAGAAGCTGTCAATTTTAATGTCACGACTGCTAGCGCCACAGATTTTTGGACGGTTGCAT
TTGCTACTTTTGTTGATGTTTTGGTTAATGTTAGTGCAACTAACATTCAAAACTTACTTT
ATTGCGATTCTCCATTTGAAAAGTTGCAGTGTGAGCACTTGCAGTTTGGATTGCAGGATG
GTTTTTATTCTGCAAATTTTCTTGATGATAATGTTTTGCCTGAGACTTATGTTGCACTCC
CCATTTATTATCAACACACGGACATAAATTTTACTGCAACTGCATCTTTTGGTGGTTCTT
GTTATGTTTGTAAACCACACCAGGTTAATATATCTCTTAATGGTAACACTTCAGTGTGTG
TTAGAACATCTCATTTTTCAATTAGGTATATTTATAACCGCGTTAAGAGTGGTTCACCAG
GTGACTCTTCATGGCACATTTATTTAAAGAGTGGCACTTGTCCATTTTCTTTTTCTAAGT
TAAATAATTTTCAAAAGTTCAAGACTATTTGTTTCTCAACCGTCGAAGTGCCTGGTAGTT
GTAATTTTCCGCTTGAAGCCACCTGGCATTACACTTCTTATACTATTGTTGGTGCTTTGT
ATGTTACTTGGTCTGAAGGTAATTCTATTACTGGTGTACCTTATCCTGTCTCTGGTATTC
GTGAGTTTAGTAATTTAGTTTTAAATAATTGTACCAAATATAATATTTATGATTATGTTG
GTACTGGAATTATACGTTCTTCAAACCAGTCACTTGCTGGTGGTATTACATATGTTTCTA
ACTCTGGTAATTTACTTGGTTTTAAAAATGTTTCCACTGGTAACATTTTTATTGTGACAC
CATGTAACCAACCAGACCAAGTAGCTGTTTATCAACAAAGCATTATTGGTGCCATGACCG
CTGTTAATGAGTCTAGATATGGCTTGCAAAACTTACTACAGTTACCTAACTTTTATTATG
TTAGTAATGGTGGTAACAATTGCACTACGGCCGTTATGACTTATTCTAATTTTGGTATTT
GTGCTGATGGTTCTTTGATTCCTGTTCGTCCGCGTAATTCTAGTGATAATGGTATTTCAG
CCATAATCACTGCTAATTTATCCATTCCTTCTAACTGGACTACTTCAGTTCAAGTTGAGT
ACCTCCAAATTACTAGTACTCCAATAGTTGTTGATTGTGCTACTTATGTGTGTAATGGTA
ACCCTCGCTGTAAGAATCTACTTAAGCAGTATACTTCTGCTTGTAAAACTATTGAAGATG

Figure 19 (Cont)

CCTTACGACTTAGTGCTCATTTGGAAACTAATGATGTTAGTAGTATGCTAACTTTCGATA
GCAATGCTTTTAGTTTGGCTAATGTTACTAGTTTTGGAGATTATAACCTTTCTAGTGTTT
TACCTCAGAGAAACATTCGTTCAAGCCGTATAGCAGGACGTAGTGCTTTGGAAGATTTGT
TGTTTAGCAAAGTTGTTACATCTGGTTTGGGTACTGTTGATGTTGACTATAAGTCTTGTA
CTAAAGGTCTTTCTATTGCTGACCTTGCTTGTGCTCAGTACTACAATGGCATAATGGTTT
TGCCAGGTGTTGCTGATGCTGAACGTATGGCCATGTACACAGGTTCTCTTATAGGTGGCA
TGGTGCTCGGAGGTCTTACATCAGCAGCCGCCATACCTTTTTCTTTGGCACTGCAAGCAC
GACTTAACTATGTTGCTTTACAAACTGATGTGCTTCAAGAAAATCAGAAAATTTTGGCTG
CATCATTTAATAAGGCTATTAATAATATTGTTGCTTCTTTTAGTAGCGTTAATGATGCTA
TTACACAAACTGCAGAGGCTATACATACTGTTACTATTGCACTTAATAAGATTCAGGATG
TTGTTAATCAACAGGGTAGTGCTCTTAACCATCTCACTTCACAATTGAGACATAATTTTC
AGGCCATTTCTAATTCAATTCAGGCTATTTATGACCGGCTTGATTCAATTCAAGCCGATC
AACAAGTTGACAGATTAATTACTGGACGGCTTGCAGCTTTGAATGCATTTGTTTCCCAAG
TTTTGAATAAATATACTGAAGTTCGTGGTTCAAGACGCTTAGCACAGCAGAAGATTAATG
AATGTGTCAAGTCACAATCTAATAGATATGGTTTTTGTGGCAATGGCACTCACATCTTTT
CAATCGTCAACTCTGCTCCAGATGGTTTGCTTTTTCTTCATACTGTTTTGCTGCCAACTG
ATTACAAGAATGTAAAGGCGTGGTCTGGTATCTGTGTTGATGGCATTTATGGCTATGTTC
TGCGTCAACCTAACTTGGTTCTTTATTCTGATAATGGTGTCTTTCGTGTAACTTCCAGGG
TCATGTTTCAACCTCGCTTACCTGTTTTGTCTGATTTTGTGCAAATATATAATTGTAATG
TTACTTTTGTTAACATATCTCGTGTTGAGTTACATACTGTCATACCTGACTACGTTGATG
TTAATAAAACATTACAAGAGTTTGCACAAAACTTACCAAAGTATGTTAAGCCTAATTTTG
ACTTGACTCCTTTTAATTTAACATATCTTAATTTGAGTTCTGAGTTGAAGCAACTCGAAG
CTAAAACTGCTAGTCTTTTTCAAACTACTGTTGAATTACAAGGTCTTATTGATCAGATTA
ACAGTACATATGTTGATTTGAAGTTGCTTAATAGGTTTGAAAATTATATCAAATGGCCTT
GGTGGGTTTGGCTCATTATTTCTGTTGTTTTTGTTGTATTGTTGAGTCTTCTTGTGTTTT
GTTGTCTTTCTACAGGTTGTTGTGGTTGTTGCAATTGTTTAACTTCATCAATGCGAGGCT
GTTGTGATTGTGGTTCAACTAAACTTCCTTATTACGAATTTGAAAAGGTCCACGTTCAAT
AATGCCTTTTGGTGGCCTATTTCAACTTACTCTTGAAAGTACTATTAATAAGAGTGTGGC
TAATCTCAAATTACCACCTCATGATGTTACTGTCTTGCGTGACAATCTTAAACCTGTTAC
TACACTTAGTACTATTACTGCTTATTTGTTAGTTAGTTTGTTTGTCACTTACTTTGCTTT
ATTCAAACCTCTTACTGCTAGAGGTCGTGTTGCTTGTTTTGTTTTAAAACTATTGACACT
ATTTGTCTATGTGCCTTTATTGGTTCTTTTTGGTATGTATCTTGACAGTTTTATAATTTT
TTCTACGCTGTTGTTTCGATTCATACATGTTGGCTATTATGCCTATCTCTATAAAAATTT
TTCATTTGTTTTGTTCAATGTTACTAAACTATGCTTCGTTTCAGGCAAGTGTTGGTATCT
TGAACAATCATTTTATGAAAATCGTTTTGCTGCTATTTATGGTGGTGACCACTATGTCGT
TTTAGGTGGTGAAACTATTACTTTTGTTTCTTTTGATGACCTTTATGTTGCTATTAGAGG
TTCTTGTGAAAAGAACCTACAACTTATGCGTAAGGTTGACTTGTATAATGGTGCTGTCAT
TTACATTTTTGCCGAAGAGCCTGTTGTTGGTATAGTCTACTCTTCTCAACTATACGAAGA
TGTTCCTTCGATTAATTGATGACAATGGTATTGTCCTCAATTCCATTTTATGGCTCCTTG
TTATGATATTTTTCTTTGTGTTGGCAATGACCTTTATTAAACTGATTCAATTGTGTTTTA
CTTGTCATTATTTTTTTAGTAGGACATTATATCAACCAGTTTATAAAATTTTTCTTGCTT
ACCAAGATTATATGCAAATAGCACCTGTTCCAGCTGAAGTACTAAATGTCTAAACTAAAC
GATGTCTAATAGTAGTGTGCCTCTTTTAGAGGTTTATGTCCATTTACGTAACTGGAACTT
TAGTTGGAATTTAATTCTAACGCTTTTTATAGTTGTGTTGCAGTATGGGCATTATAAGTA
TAGCAGACTTCTTTATGGTTTAAAGATGTCTGTTTTATGGTGTTTATGGCCACTTGTTCT
AGCTTTGTCTATTTTTGACTGTTTTGTCAATTTTAATGTGGACTGGGTCTTTTTTGGTTT
TAGTATTCTTATGTCTATTATTACACTTTGTTTATGGGTTATGTATTTTGTTAATAGTTT
CAGACTTTGGCGCCGTGTTAAAACTTTTTGGGCTTTTAATCCTGAAACTAATGCAATCAT
CTCTCTCCAGGTTTACGGACATAATTATTACTTACCGGTGATGGCTGCACCTACAGGTGT
TACATTAACACTTCTTAGTGGTGTACTTCTTGTTGATGGCCATAAGATTGCTACTCGTGT
TCAAGTGGGTCAGTTGCCTAAATATGTAATAGTTGCTACGCCTAGTACCACAATTGTTTG
TGACCGTGTTGGTCGCTCTGTTAATGAAACAAGCCAGACTGGTTGGGCATTCTACGTCCG
TGCTAAACATGGTGATTTTTCTGGTGTTGCCTCTCAGGAGGGTGTTTTGTCAGAAAGAGA
GAAGTTGCTTCATTTAATCTAAACTAAACAAAATGGCTAGTGTAAATTGGGCCGATGACA
GAGCTGCTAGGAAGAAATTTCCTCCTCCTTCATTTTACATGCCTCTTTTGGTTAGTTCTG

Figure 19 (Cont)

ATAAGGCACCATATAGGGTCATTCCCAGGAATCTTGTCCCTATTGGTAAGGGTAATAAAG
ATGAGCAGATTGGTTATTGGAATGTTCAAGAGCGTTGGCGTATGCGCAGGGGGCAACGTG
TTGATTTGCCTCCTAAAGTTCATTTTTATTACCTAGGTACTGGACCTCATAAGGACCTTA
AATTCAGACAACGTTCTGATGGTGTTGTTTGGGTTGCTAAGGAAGGTGCTAAAACTGTTA
ATACCAGTCTTGGTAATCGCAAACGTAATCAGAAACCTTTGGAACCAAAGTTCTCTATTG
CTTTGCCTCCAGAGCTCTCTGTTGTTGAGTTTGAGGATCGCTCTAATAACTCATCTCGTG
CTAGCAGTCGTTCTTCAACTCGTAACAACTCACGAGACTCTTCTCGTAGCACTTCAAGAC
AACAGTCTCGCACTCGTTCTGATTCTAACCAGTCTTCTTCAGATCTTGTTGCTGCTGTTA
CTTTGGCCTTAAAGAACTTAGGTTTTGATAACCAGTCGAAGTCACCTAGTTCTTCTGGTA
CTTCCACTCCTAAGAAACCTAATAAGCCTCTTTCTCAACCCAGGGCTGATAAGCCTTCTC
AGTTGAAGAAACCTCGTTGGAAGCGTGTTCCTACCAGAGAGGAAAATGTTATTCAGTGCT
TTGGTCCTCGTGATTTTAATCACAATATGGGGGATTCAGATCTTGTTCAGAATGGTGTTG
ATGCCAAAGGTTTTCCACAGCTTGCTGAATTGATTCCTAATCAGGCTGCGTTATTCTTTG
ATAGTGAGGTTAGCACTGATGAAGTGGGTGATAATGTTCAGATTACCTACACCTACAAAA
TGCTTGTAGCTAAGGATAATAAGAACCTTCCTAAGTTCATTGAGCAGATTAGTGCTTTTA
CTAAACCCAGTTCTATCAAAGAAATGCAGTCACAATCATCTCATGTTGCTCAGAACACAG
TACTTAATGCTTCTATTCCAGAATCTAAACCATTGGCTGATGATGATTCAGCCATTATAG
AAATTGTCAACGAGGTTTTGCATTAAATTGTTTTGTAATTCCAGTTGAATGTTTATTATT
ATTAGTTGCAACCCCATGCGTTTAGCGCATGATAAGGGTTTAGTCTTACACACAATGGTA
GGCCAGTGATAGTAAAGTGTAAGTAATTTGCTATCATATTAACATGTCTAGAGGAAAGTC
AGAACTTTTTCTGTTTGTGTTGTTGGAGTACTTAAAGATCGCATAGGCGCGCCAACAATG
GAAGAGCCAACAACATATCTAAAAATGTTTTGTCTGGTACTTGTTAATGATATTGTTTTT
GATATGGATACAC

Figure 20

ORF 1a, replicase enzyme complex

MFYNQVTLAVASDSEISGFGFAIPSVAVRTYSEAAAQGFQACRFVAFGLQDCVTGINDDD
YVIALTGTNQLCAKILPFSDRPLNLRGWLIFSNSNYVLQDFDVVFGHGAGSVVFVDKYMC
GFDGKPVLPKNMWEFRDYFNNNTDSIVIGGVTYQLAWDVIRKDLSYEQQNVLAIESIHYL
GTTGHTLKSGCKLTNAKPPKYSSKVVLSGEWNAVYRAFGSPFITNGMSLLDIIVKPVFFN
AFVKCNCGSESWSVGAWDGYLSSCCGTPAKKLCVVPGNVVPGDVIITSTSAGCGVKYYAG
LVVKHITNITGVSLWRVTAVHSDGMFVASSSYDALLHRNSLDPFCFDVNTLLSNQLRLAF
LGASVTEDVKFAASTGVIDISAGMFGLYDDILTNNKPWFVRKASGLFDAIWDAFVAAIKL
VPTTTGVLVRFVKSIASTVLTVSNGVIIMCADVPDAFQSVYRTFTQAICAAFDFSLDVFK
IGDVKFKRLGDYVLTENALVRLTTEVVRGVRDARIKKAMFTKVVVGPTTEVKFSVIELAT
VNLRLVDCAPVVCPKGKIVVIAGQAFFYSGGFYRFMVDPTTVLNDPVFTGDLFYTIKFSG
FKLDGFNHQFVTASSATDAIIAVELLLLDFKTAVFVYTCVVDGCSVIVRRDATFATHVCF
KDCYNVWEQFCIDNCGEPWFLTDYNAILQSNNPQCAIVQASESKVLLERFLPKCPEILLS
IDDGHLWNLFVEKFNFVTDWLKTLKLTLTSNGLLGNCAKRFRRVLVKLLDVYNGFLETVC
SVAYTAGVCIKYYAVNVPYVVISGFVSRVIRRERCDMTFPCVSCVTFFYEFLDTCFGVSK
PNAIDVEHLELKETVFVEPKDGGQFFVSGDYLWYVVDDIYYPASCNGVLPVAFTKLAGGK
ISFSDDVIVHDVEPTHKVKLIFEFEDDVVTSLCKKSFGKSIIYTGDWEGLHEVLTSAMNV
IGQHIKLPQFYIYDEEGGYDVSKPVMISQWPISNDSNGCVVEASTDFHQLECIVDDSVRE
EVDIIEQPFEEVEHVLSIKQPFSFSFRDELGVRVLDQSDNNCWISTTLVQLQLTKLLDDS
IEMQLFKVGKVDSIVQKCYELSHLISGSLGDSGKLLSELLKEKYTCSITFEMSCDCGKKF
DDQVGCLFWIMPYTKLFQKGECCICHKMQTYKLVSMKGTGVFVQDPAPIDIDAFPVKPIC
SSVYLGVKGSGHYQTNLYSFNKAIDGFGVFDIKNSSVNTVCFVDVDFHSVEIEAGEVKPF
AVYKNVKFYLGDISHLVNCVSFDFVVNAANENLLHGGGVARAIDILTEGQLQSLSKDYIS
SNGPLKVGAGVMLECEKFNVFNVVGPRTGKHEHSLLVEAYNSILFENGIPLMPLLSCGIF
GVRIENSLKALFSCDINKPLQVFVYSSNEEQAVLKFLDGLDLTPVIDDVDVVKPFRVEGN
FSFFDCGVNALDGDIYLLFTNSILMLDKQGQLLDTKLNGILQQAALDYLATVKTVPAGNL
VKLFVESCTIYMCVVPSINDLSFDKNLGRCVRKLNRLKTCVIANVPAIDVLKKLLSSLTL
TVKFVVESNVMDVNDCFKNDNVVLKITEDGINVKDVVVESSKSLGKQLGVVSDGVDSFEG
VLPINTDTVLSVAPEVDWVAFYGFEKAALFASLDVKPYGYPNDFVGGFRVLGTTDNNCWV
NATCIILQYLKPTFKSKGLNVLWNKFVTGDVGPFVSFIYFITMSSKGQKGDAEEALSKLS
EYLISDSIVTLEQYSTCDICKSTVVEVKSAIVCASVLKDGCDVGFCPHRHKLRSRVKFVN
GRVVITNVGEPIISQPSKLLNGIAYTTFSGSFDNGHYVVYDAANNAVYDGARLFSSDLST
LAVTAIVVVGGCVTSNVPTIVSEKISVMDKLDTGAQKFFQFGDFVMNNIVLFLTWLLSMF
SLLRTSIMKHDIKVIAKAPKRTGVILTRSFKYNIRSALFVIKQKWCVIVTLFKFLLLLYA
IYALVFMIVQFSPFNSLLCGDIVSGYEKSTFNKDIYCGNSMVCKMCLFSYQEFNDLDHTS
LVWKHIRDPILISLQPFVILVILLIFGNMYLRFGLLYFVAQFISTFGSFLGFHQKQWFLH
FVPFDVLCNEFLATFIVCKIVLFVRHIIVGCNNADCVACSKSARLKRVPLQTIINGMHKS
FYVNANGGTCFCNKHNFFCVNCDSFGPGNTFINGDIARELGNVVKTAVQPTAPAYVIIDK
VDFVNGFYRLYSGDTFWRYDFDITESKYSCKEVLKNCNVLENFIVYNNSGSNITQIKNAC
VYFSQLLCEPIKLVNSELLSTLSVDFNGVLHKAYVDVLCNSFFKELTANMSMAECKATLG
LTVSDDDFVSAVANAHRYDVLLSDLSFNNFFISYAKPEDKLSVYDIACCMRAGSKVVNHN
VLIKESIPIVWGVKDFNTLSQEGKKYLVKTTKAKGLTFLLTFNDNQAITQVPATSIVAKQ
GAGFKRTYNFLWYVCLFVVALFIGVSFIDYTTTVTSFHGYDFKYIENGQLKVFEAPLHCV
RNVFDNFNQWHEAKFGVVTTNSDKCPIVVGVSERINVVPGVPTNVYLVGKTLVFTLQAAF
GNTGVCYDFDGVTTSDKCIFNSACTRLEGLGGDNVYCYNTDLIEGSKPYSTLQPNAYYKY
DAKNYVRFPEILARGFGLRTIRTLATRYCRVGECRDSHKGVCFGFDKWYVNDGRVDDGYI
CGDGLIDLLVNVLSIFSSSFSVVAMSGHMLFNFLFAAFITFLCFLVTKFKRVFGDLSYGV
FTVVCATLINNISYVVTQNLFFMLLYAILYFVFTRTVRYAWIWHIAYIVAYFLLIPWWLL
TWFSFAAFLELLPNVFKLKISTQLFEGDKFIGTFESAAAGTFVLDMRSYERLINTISPEK
LKNYAASYNKYKYYSGSASEADYRCACYAHLAKAMLDYAKDHNDMLYSPPTISYNSTLQS
GLKKMAQPSGCVERCVVRVCYGSTVLNGVWLGDTVTCPRHVIAPSTTVLIDYDHAYSTMR
LHNFSVSHNGVFLGVVGVTMHGSVLRIKVSQSNVHTPKHVFKTLKPGDSFNILACYEGIA
SGVFGVNLRTNFTIKGSFINGACGSPGYNVRNDGTVEFCYLHQIELGSGAHVGSDFTGSV

Figure 20 (Cont.)

YGNFDDQPSLQVESANLMLSDNVVAFLYAALLNGCRWWLCSTRVNVDGFNEWAMANGYTS
VSSVECYSILAAKTGVSVEQLLASIQHLHEGFGGKNILGYSSLCDEFTLAEVVKQMYGVN
LQSGKVIFGLKTMFLFSVFFTMFWAELFIYTNTIWINPVILTPIFCLLLFLSLVLTMFLK
HKFLFLQVFLLPTVIATALYNCVLDYYIVKFLADHFNYNVSVLQMDVQGLVNVLVCLFVV
FLHTWRFSKERFTHWFTYVCSLIAVAYTYFYSGDFLSLLVMFLCAISSDWYIGAIVFRLS
RLIVFFSPESVFSVFGDVKLTLVVYLICGYLVCTYWGILYWFNRFFKCTMGVYDFKVSAA
EFKYMVANGLHAPHGPFDALWLSFKLLGIGGDRCIKISTVQSKLTDLKCTNVVLLGCLSS
MNIAANSSEWAYCVDLHNKINLCDDPEKAQSMLLALLAFFLSKHSDFGLDGLIDSYFDNS
STLQSVASSFVSMPSYIAYENARQAYEDAIANGSSSQLIKQLKRAMNIAKSEFDHEISVQ
KKINRMAEQAATQMYKEARSVNRKSKVISAMHSLLFGMLRRLDMSSVETVLNLARDGVVP
LSVIPATSASKLTIVSPDLESYSKIVCDGSVHYAGVVWTLNDVKDNDGRPVHVKEITKEN
VETLTWPLILNCERVVKLQNNEIMPGKLKQKPMKAEGDGGVLGDGNALYNTEGGKTFMYA
YISNKADLKFVKWEYEGGCNTIELDSPCRFMVETPNGPQVKYLYFVKNLNTLRRGAVLGF
IGATIRLQAGKQTELAVNSGLLTACAFSVDPATTYLEAVKHGAKPVSNCIKMLSNGAGNG
QAITTSVDANTNQDSYGGASICLYCRAHVPHPSMDGYCKFKGKCVQVPIGCLDPIRFCLE
NNVCNVCGCWLGHGCACDRTTIQSVDISYLNEQGVLVQLD

Figure 21

ORF 1ab replicase polyprotein

MFYNQVTLAVASDSEISGFGFAIPSVAVRTYSEAAAQGFQACRFVAFGLQDCVTGINDDD
YVIALTGTNQLCAKILPFSDRPLNLRGWLIFSNSNYVLQDFDVVFGHGAGSVVFVDKYMC
GFDGKPVLPKNMWEFRDYFNNNTDSIVIGGVTYQLAWDVIRKDLSYEQQNVLAIESIHYL
GTTGHTLKSGCKLTNAKPPKYSSKVVLSGEWNAVYRAFGSPFITNGMSLLDIIVKPVFFN
AFVKCNCGSESWSVGAWDGYLSSCCGTPAKKLCVVPGNVVPGDVIITSTSAGCGVKYYAG
LVVKHITNITGVSLWRVTAVHSDGMFVASSSYDALLHRNSLDPFCFDVNTLLSNQLRLAF
LGASVTEDVKFAASTGVIDISAGMFGLYDDILTNNKPWFVRKASGLFDAIWDAFVAAIKL
VPTTTGVLVRFVKSIASTVLTVSNGVIIMCADVPDAFQSVYRTFTQAICAAFDFSLDVFK
IGDVKFKRLGDYVLTENALVRLTTEVVRGVRDARIKKAMFTKVVVGPTTEVKFSVIELAT
VNLRLVDCAPVVCPKGKIVVIAGQAFFYSGGFYRFMVDPTTVLNDPVFTGDLFYTIKFSG
FKLDGFNHQFVTASSATDAIIAVELLLLDFKTAVFVYTCVVDGCSVIVRRDATFATHVCF
KDCYNVWEQFCIDNCGEPWFLTDYNAILQSNNPQCAIVQASESKVLLERFLPKCPEILLS
IDDGHLWNLFVEKFNFVTDWLKTLKLTLTSNGLLGNCAKRFRRVLVKLLDVYNGFLETVC
SVAYTAGVCIKYYAVNVPYVVISGFVSRVIRRERCDMTFPCVSCVTFFYEFLDTCFGVSK
PNAIDVEHLELKETVFVEPKDGGQFFVSGDYLWYVVDDIYYPASCNGVLPVAFTKLAGGK
ISFSDDVIVHDVEPTHKVKLIFEFEDDVVTSLCKKSFGKSIIYTGDWEGLHEVLTSAMNV
IGQHIKLPQFYIYDEEGGYDVSKPVMISQWPISNDSNGCVVEASTDFHQLECIVDDSVRE
EVDIIEQPFEEVEHVLSIKQPFSFSFRDELGVRVLDQSDNNCWISTTLVQLQLTKLLDDS
IEMQLFKVGKVDSIVQKCYELSHLISGSLGDSGKLLSELLKEKYTCSITFEMSCDCGKKF
DDQVGCLFWIMPYTKLFQKGECCICHKMQTYKLVSMKGTGVFVQDPAPIDIDAFPVKPIC
SSVYLGVKGSGHYQTNLYSFNKAIDGFGVFDIKNSSVNTVCFVDVDFHSVEIEAGEVKPF
AVYKNVKFYLGDISHLVNCVSFDFVVNAANENLLHGGGVARAIDILTEGQLQSLSKDYIS
SNGPLKVGAGVMLECEKFNVFNVVGPRTGKHEHSLLVEAYNSILFENGIPLMPLLSCGIF
GVRIENSLKALFSCDINKPLQVFVYSSNEEQAVLKFLDGLDLTPVIDDVDVVKPFRVEGN
FSFFDCGVNALDGDIYLLFTNSILMLDKQGQLLDTKLNGILQQAALDYLATVKTVPAGNL
VKLFVESCTIYMCVVPSINDLSFDKNLGRCVRKLNRLKTCVIANVPAIDVLKKLLSSLTL
TVKFVVESNVMDVNDCFKNDNVVLKITEDGINVKDVVVESSKSLGKQLGVVSDGVDSFEG
VLPINTDTVLSVAPEVDWVAFYGFEKAALFASLDVKPYGYPNDFVGGFRVLGTTDNNCWV
NATCIILQYLKPTFKSKGLNVLWNKFVTGDVGPFVSFIYFITMSSKGQKGDAEEALSKLS
EYLISDSIVTLEQYSTCDICKSTVVEVKSAIVCASVLKDGCDVGFCPHRHKLRSRVKFVN
GRVVITNVGEPIISQPSKLLNGIAYTTFSGSFDNGHYVVYDAANNAVYDGARLFSSDLST
LAVTAIVVVGGCVTSNVPTIVSEKISVMDKLDTGAQKFFQFGDFVMNNIVLFLTWLLSMF
SLLRTSIMKHDIKVIAKAPKRTGVILTRSFKYNIRSALFVIKQKWCVIVTLFKFLLLLYA
IYALVFMIVQFSPFNSLLCGDIVSGYEKSTFNKDIYCGNSMVCKMCLFSYQEFNDLDHTS
LVWKHIRDPILISLQPFVILVILLIFGNMYLRFGLLYFVAQFISTFGSFLGFHQKQWFLH
FVPFDVLCNEFLATFIVCKIVLFVRHIIVGCNNADCVACSKSARLKRVPLQTIINGMHKS
FYVNANGGTCFCNKHNFFCVNCDSFGPGNTFINGDIARELGNVVKTAVQPTAPAYVIIDK
VDFVNGFYRLYSGDTFWRYDFDITESKYSCKEVLKNCNVLENFIVYNNSGSNITQIKNAC
VYFSQLLCEPIKLVNSELLSTLSVDFNGVLHKAYVDVLCNSFFKELTANMSMAECKATLG
LTVSDDDFVSAVANAHRYDVLLSDLSFNNFFISYAKPEDKLSVYDIACCMRAGSKVVNHN
VLIKESIPIVWGVKDFNTLSQEGKKYLVKTTKAKGLTFLLTFNDNQAITQVPATSIVAKQ
GAGFKRTYNFLWYVCLFVVALFIGVSFIDYTTTVTSFHGYDFKYIENGQLKVFEAPLHCV
RNVFDNFNQWHEAKFGVVTTNSDKCPIVVGVSERINVVPGVPTNVYLVGKTLVFTLQAAF
GNTGVCYDFDGVTTSDKCIFNSACTRLEGLGGDNVYCYNTDLIEGSKPYSTLQPNAYYKY
DAKNYVRFPEILARGFGLRTIRTLATRYCRVGECRDSHKGVCFGFDKWYVNDGRVDDGYI
CGDGLIDLLVNVLSIFSSSFSVVAMSGHMLFNFLFAAFITFLCFLVTKFKRVFGDLSYGV
FTVVCATLINNISYVVTQNLFFMLLYAILYFVFTRTVRYAWIWHIAYIVAYFLLIPWWLL
TWFSFAAFLELLPNVFKLKISTQLFEGDKFIGTFESAAAGTFVLDMRSYERLINTISPEK
LKNYAASYNKYKYYSGSASEADYRCACYAHLAKAMLDYAKDHNDMLYSPPTISYNSTLQS
GLKKMAQPSGCVERCVVRVCYGSTVLNGVWLGDTVTCPRHVIAPSTTVLIDYDHAYSTMR
LHNFSVSHNGVFLGVVGVTMHGSVLRIKVSQSNVHTPKHVFKTLKPGDSFNILACYEGIA
SGVFGVNLRTNFTIKGSFINGACGSPGYNVRNDGTVEFCYLHQIELGSGAHVGSDFTGSV

Figure 21 (Cont.)

YGNFDDQPSLQVESANLMLSDNVVAFLYAALLNGCRWWLCSTRVNVDGFNEWAMANGYTS
VSSVECYSILAAKTGVSVEQLLASIQHLHEGFGGKNILGYSSLCDEFTLAEVVKQMYGVN
LQSGKVIFGLKTMFLFSVFFTMFWAELFIYTNTIWINPVILTPIFCLLLFLSLVLTMFLK
HKFLFLQVFLLPTVIATALYNCVLDYYIVKFLADHFNYNVSVLQMDVQGLVNVLVCLFVV
FLHTWRFSKERFTHWFTYVCSLIAVAYTYFYSGDFLSLLVMFLCAISSDWYIGAIVFRLS
RLIVFFSPESVFSVFGDVKLTLVVYLICGYLVCTYWGILYWFNRFFKCTMGVYDFKVSAA
EFKYMVANGLHAPHGPFDALWLSFKLLGIGGDRCIKISTVQSKLTDLKCTNVVLLGCLSS
MNIAANSSEWAYCVDLHNKINLCDDPEKAQSMLLALLAFFLSKHSDFGLDGLIDSYFDNS
STLQSVASSFVSMPSYIAYENARQAYEDAIANGSSSQLIKQLKRAMNIAKSEFDHEISVQ
KKINRMAEQAATQMYKEARSVNRKSKVISAMHSLLFGMLRRLDMSSVETVLNLARDGVVP
LSVIPATSASKLTIVSPDLESYSKIVCDGSVHYAGVVWTLNDVKDNDGRPVHVKEITKEN
VETLTWPLILNCERVVKLQNNEIMPGKLKQKPMKAEGDGGVLGDGNALYNTEGGKTFMYA
YISNKADLKFVKWEYEGGCNTIELDSPCRFMVETPNGPQVKYLYFVKNLNTLRRGAVLGF
IGATIRLQAGKQTELAVNSGLLTACAFSVDPATTYLEAVKHGAKPVSNCIKMLSNGAGNG
QAITTSVDANTNQDSYGGASICLYCRAHVPHPSMDGYCKFKGKCVQVPIGCLDPIRFCLE
NNVCNVCGCWLGHGCACDRTTIQSVDISYLNEQGVLVQLDRARGSSAARLEPCNGTDIDK
CVRAFDIYNKNVSFLGKCLKMNCVRFKNADLKDGYFVIKRCTKSVMEHEQSMYNLLNFSG
ALAEHDFFTWKDGRVIYGNVSRHNLTKYTMMDLVYAMRNFDEQNCDVLKEVLVLTGCCDN
SYFDSKGWYDPVENEDIHRVYASLGKIVARAMLKCVALCDAMVAKGVVGVLTLDNQDLNG
NFYDFGDFVVSLPNMGVPCCTSYYSYMMPIMGLTNCLASECFVKSDIFGSDFKTFDLLKY
DFTEHKENLFNKYFKHWSFDYHPNCCDCYDDMCVIHCANFNTLFATTIPGTAFGPLCRKV
FIDGVPLVTTAGYHFKQLGLVWNKDVNTHSVRLTITELLQFVTDPSLIIASSPALVDQRT
ICFSVAALSTGLTNQVVKPGHFNEEFYNFLRLRGFFDEGSELTLKHFFFAQNGDAAVKDF
DFYRYNKPTILDICQARVTYKIVSRYFDIYEGGCIKACEVVVTNLNKSAGWPLNKFGKAS
LYYESISYEEQDALFALTKRNVLPTMTQLNLKYAISGKERARTVGGVSLLSTMTTRQYHQ
KHLKSIVNTRNATVVIGTTKFYGGWNNMLRTLIDGVENPMLMGWDYPKCDRALPNMIRMI
SAMVLGSKHVNCCTATDRFYRLGNELAQVLTEVVYSNGGFYFKPGGTTSGDASTAYANSI
FNIFQAVSSNINRLLSVPSDSCNNVNVRDLQRRLYDNCYRLTSVEESFIEDYYGYLRKHF
SMMILSDDGVVCYNKDYAELGYIADISAFKATLYYQNNVFMSTSKCWVEEDLTKGPHEFC
SQHTMQIVDKDGTYYLPYPDPSRILSAGVFVDDVVKTDAVVLLERYVSLAIDAYPLSKHP
NSEYRKVFYVLLDWVKHLNKNLNEGVLESFSVTLLDNQEDKFWCEDFYASMYENSTILQA
AGLCVVCGSQTVLRCGDCLRKPMLCTKCAYDHVFGTDHKFILAITPYVCNASGCGVSDVK
KLYLGGLNYYCTNHKPQLSFPLCSAGNIFGLYKNSATGSLDVEVFNRLATSDWTDVRDYK
LANDVKDTLRLFAAETIKAKEESVKSSYAFATLKEVVGPKELLLSWESGKVKPPLNRNSV
FTCFQISKDSKFQIGEFIFEKVEYGSDTVTYKSTVTTKLVPGMIFVLTSHNVQPLRAPTI
ANQEKYSSIYKLHPAFNVSDAYANLVPYYQLIGKQKITTIQGPPGSGKSHCSIGLGLYYP
GARIVFVACAHAAVDSLCAKAMTVYSIDKCTRIIPARARVECYSGFKPNNTSAQYIFSTV
NALPECNADIVVVDEVSMCTNYDLSVINQRLSYKHIVYVGDPQQLPAPRVMITKGVMEPV
DYNVVTQRMCAIGPDVFLHKCYRCPAEIVIQFLNLFMRTSLSLLNLLVNSVLKSFLRVMY
KVDNGSSINRKQLEIVKLFLVKNPSWSKAVFISPYNSQNYVASRFLGLQIQTVDSSQGSE
YDYVIYAQTSDTAHACNVNRFNVAITRAKKGIFCVMCDKTLFDSLKFFEIKHADLHSSQV
CGLFKNCTRTPLNLPPTHAHTFLSLSDQFKTTGDLAVQIGSNNVCTYEHVISFMGFRFDI
SIPGSHSLFCTRDFAIRNVRGWLGMDVESAHVCGDNIGTNVPLQVGFSNGVNFVVQTEGC
VSTNFGDVIKPVCAKSPPGEQFRHLIPLLRKGQPWLIVRRRIVQMISDYLSNLSDILVFV
LWAGSLELTTMRYFVKIGPIKYCYCGNFATCYNSVSNEYCCFKHALGCDYVYNPYAFDIQ
QWGYVGSLSQNHHTFCNIHRNEHDASGDAVMTRCLAVHDCFVKNVDWTVTYPFIANEKFI
NGCGRNVQGHVVRAALKLYKPSVIHDIGNPKGVRCAVTDAKWYCYDKQPVNSNVKLLDYD
YATHGQLDGLCLFWNCNVDMYPEFSIVCRFDTRTRSVFNLEGVNGGSLYVNKHAFHTPAY
DKRAFVKLKPMPFFYFDDSDCDVVQEQVNYVPLRASSCVTRCNIGGAVCSKHANLYQKYV
EAYNTFTQAGFNIWVPHSFDVYNLWQIFIETNLQSLENIAFNVVKKGCFTGVDGELPVAV
VNDKVFVRYGDVDNLVFTNKTTLPTNVAFELFAKRKMGLTPPLSILKNLGVVATYKFVLW
DYEAERPFTSYTKSVCKYTDFNEDVCVCFDNSIQGSYERFTLTTNAVLFSTVVIKNLTPI
KLNFGMLNGMPVSSIKGDKGVEKLVNWYIYVRKNGQFQDHYDGFYTQGRNLSDFTPRSDM
EYDFLNMDMGVFINKYGLEDFNFEHVVYGDVSKTTLGGLHLLISQFRLSKMGVLKADDFV

Figure 21 (Cont.)

TASDTTLRCCTVTYLNELSSKVVCTYMDLLLDDFVTILKSLDLGVISKVHEVIIDNKPYR
WMLWCKDNHLSTFYPQLQSAEWKCGYAMPQIYKLQRMCLEPCNLYNYGAGIKLPSGIMLN
VVKYTQLCQYLNSTTMCVPHNMRVLHYGAGSDKGVAPGTTVLKRWLPPDAIIIDNDINDY
VSDADFSITGDCATVYLEDKFDLLISDMYDGRIKFCDGENVSKDGFFTYLNGVIREKLAI
GGSVAIKITEYSWNKYLYELIQRFAFWTLFCTSVNTSSSEAFLIGINYLGDFIQGPFIAG
NTVHANYIFWRNSTIMSLSYNSVLDLSKFECKHKATVVVTLKDSDVNDMVLSLIKSGRLL
LRNNGRFGGFSNHLVSTK

Like the ORF 1a gene product, this polyprotein is proteolytically cleaved at sites corresponding to the consensus **LQ↓(S, G or A)**[1] by action of the $3Cl^{pro}$ protease. Potential proteolytic cleavage sites are indicated in bold print in grey background. The $3Cl^{pro}$-encoding domain is boldly underlined and is also shown as separate protein below. The remaining proteolysis products perform functions in the replication and processing of the viral RNA. Normal blast searches are not sensitive enough to detect this kind of dispersed homology. However, using PFAM (**Protein Family**) domains tentative functions can be attributed to the proteolysis products.

MFYNQVTLAVASDSEISGFGFAIPSVAVRTYSEAAAQGFQACRFVAFGLQDCVTGINDDDYV
IALTGTNQLCAKILPFSDRPLNLRGWLIFSNSNYYVLQDFDVVFGHGAGSVVFVDKYMCGFDG
KPVLPKNMWEFRDYFNNNTDSIVIGGVTYQLAWDVIRKDLSYEQQNVLAIESIHYLGTTGHTL
KSGCKLTNAKPPKYSSKVVLSGEWNAVYRAFGSPFITNGMSLLDIIVKPVFFNAFVKCNCGS
ESWSVGAWDGYLSSCCGTPAKKLCVVPGNVVPGDVIITSTSAGCGVKYYAGLVVKHITNITG
VSLWRVTAVHSDGMFVASSSYDALLHRNSLDPFCFDVNTLLSNQLRLAFLGASVTEDVKFA
ASTGVIDISAGMFGLYDDILTNNKPWFVRKASGLFDAIWDAFVAAIKLVPTTTGVLVRFVKSIA
STVLTVSNGVIIMCADVPDAFQSVYRTFTQAICAAFDFSLDVFKIGDVKFKRLGDYVLTENAL
VRLTTEVVRGVRDARIKKAMFTKVVVGPTTEVKFSVIELATVNLRLVDCAPVVCPKGKIVVIA
GQAFFYSGGFYRFMVDPTTVLNDPVFTGDLFYTIKFSGFKLDGFNHQFVTASSATDAIIAVEL
LLLDFKTAVFVYTCVVDGCSVIVRRDATFATHVCFKDCYNVWEQFCIDNCGEPWFLTDYNAI
LQSNNPQCAIVQASESKVLLERFLPKCPEILLSIDDGHLWNLFVEKFNFVTDWLKTLKLTLTS
NGLLGNCAKRFRRVLVKLLDVYNGFLETVCSVAYTAGVCIKYYAVNVPYVVISGFVSRVIRRE
RCDMTFPCVSCVTFFYEFLDTCFGVSKPNAIDVEHLELKETVFVEPKDGGQFFVSGDYLWY
VVDDIYYPASCNGVLPVAFTKLAGGKISFSDDVIVHDVEPTHKVKLIFEFEDDVVTSLCKKSF
GKSIIYTGDWEGLHEVLTSAMNVIGQHIKLPQFYIYDEEGGYDVSKPVMISQWPISNDSNGC
VVEASTDFHQLECIVDDSVREEVDIIEQPFEEVEHVLSIKQPFSFSFRDELGVRVLDQSDNNC
WISTTLVQLQLTKLLDDSIEMQLFKVGKVDSIVQKCYELSHLISGSLGDSGKLLSELLKEKYTC
SITFEMSCDCGKKFDDQVGCLFWIMPYTKLFQKGECCICHKMQTYKLVSMKGTGVFVQDPA
PIDIDAFPVKPICSSVYLGVKGSGHYQTNLYSFNKAIDGFGVFDIKNSSVNTVCFVDVDFHSV
EIEAGEVKPFAVYKNVKFYLGDISHLVNCVSFDF**VVNAANEN**LLHGGGVARAIDILTEGQLQ
SLSKDYISSNGPLKVGAGVMLECEKFNVFNVVGPRTGKHEHSLLVEAYNSILFENGIPLMPL
LSCGIFGVRIENSLKALFSCDINKPLQVFVYSSNEEQAVLKFLDGLDLTPVIDDVDVVKPFRVE
GNFSFFDCGVNALDGDIYLLFTNSILMLDKQGQLLDTKLNGILQQAALDYLATVKTVPAGNLV
KLFVESCTIYMCVVPSINDLSFDKNLGRCVRKLNRLKTCVIANVPAIDVLKKLLSSLTLTVKFV
VESNVMDVNDCFKNDNVVLKITEDGINVKDVVVESSKSLGKQLGVVSDGVDSFEGVLPINTD
TVLSVAPEVDWVAFYGFEKAALFASLDVKPYGYPNDFVGGFRVLGTTDNNCWVNATCIILQ
YLKPTFKSKGLNVLWNKFVTGDVGPFVSFIYFITMSSKGQKGDAEEALSKLSEYLISDSIVTLE
QYSTCDICKSTVVEVKSAIVCASVLKDGCDVGFCPHRHKLRSRVKFVNGRVVITNVGEPIISQ
PSKLLNGIAYTTFSGSFDNGHYVVYDAANNAVYDGARLFSSDLSTLAVTAIVVVGGCVTSNV
PTIVSEKISVMDKLDTGAQKFFQFGDFVMNNIVLFLTWLLSMFSLLRTSIMKHDIKVIAKAPKR
TGVILTRSFKYNIRSALFVIKQKWCVIVTLFKFLLLLYAIYALVFMIVQFSPFNSLLCGDIVSGYE
KSTFNKDIYCGNSMVCKMCLFSYQEFNDLDHTSLVWKHIRDPILISLQPFVILVILLIFGNMYL
RFGLLYFVAQFISTFGSFLGFHQKQWFLHFVPFDVLCNEFLATFIVCKIVLFVRHIIVGCNNAD
CVACSKSARLKRVPLQTIINGMHKSFYVNANGGTCFCNKHNFFCVNCDSFGPGNTFINGDIA
RELGNVVKTAVQPTAPAYVIIDKVDFVNGFYRLYSGDTFWRYDFDITESKYSCKEVLKNCNV
LENFIVYNNSGSNITQIKNACVYFSQLLCEPIKLVNSELLSTLSVDFNGVLHKAYVDVLCNSF

Figure 21 (Cont.)

KELTANMSMAECKATLGLTVSDDDFVSAVANAHRYDVLLSDLSFNNFFISYAKPEDKLSVYD
IACCMRAGSKVVNHNVLIKESIPIVWGVKDFNTLSQEGKKYLVKTTKAKGLTFLLTFNDNQAI
TQVPATSIVAKQGAGFKRTYNFLWYVCLFVVALFIGVSFIDYTTTVTSFHGYDFKYIENGQLK
VFEAP

LHCVRNVFDNFNQWHEAKFGVVTTNSDKCPIVVGVSERINVVPGVPTNVYLVGKTLVFTLQAAFGNTG
VCYDFDGVTTSDKCIFNSACTRLEGLGGDNVYCYNTDLIEGSKPYSTLQPNAYYKYDAKNYVRFPEIL
ARGFGLRTIRTLATRYCRVGECRDSHKGVCFGFDKWYVNDGRVDDGYICGDGLIDLLVNVLSIFSSSF
SVVAMSGHMLFNFLFAAFITFLCFLVTKFKRVFGDLSYGVFTVVCATLINNISYVVTQNLFFMLLYAI
LYFVFTRTVRYAWIWHIAYIVAYFLLIPWWLLTWFSFAAFLELLPNVFKLKISTQLFEGDKFIGTFES
AAAGTFVLDMRSYERLINTISPEKLKNYAASYNKYKYYSGSASEADYRCACYAHLAKAMLDYAKDHND
MLYSPPTISYNSTLQSGLKKMAQPSGCVERCVVRVCYGSTVLNGVWLGDTVTCPRHVIAPSTTVLIDY
DHAYSTMRLHNFSVSHNGVFLGVVGVTMHGSVLRIKVSQSNVHTPKHVFKTLKPGDSFNILACYEGIA
SGVFGVNLRTNFTIKGSFINGACGSPGYNVRNDGTVEFCYLHQIELGSGAHVGSDFTGSVYGNFDDQP
SLQVESANLMLSDNVVAFLYAALLNGCRWWLCSTRVNVDGFNEWAMANGYTSVSSVECYSILAAKTGV
SVEQLLASIQHLHEGFGGKNILGYSSLCDEFTLAEVVKQMYGVNLQSGKVIFGLKTMFLFSVFFTMFW
AELFIYTNTIWINPVILTPIFCLLLFLSLVLTMFLKHKFLFLQVFLLPTVIATALYNCVLDYYIVKFL
ADHFNYNVSVLQMDVQGLVNVLVCLFVVFLHTWRFSKERFTHWFTYVCSLIAVAYTYFYSGDFLSLLV
MFLCAISSDWYIGAIVFRLSRLIVFFSPESVFSVFGDVKLTLVVYLICGYLVCTYWGILYWFNRFFKC
TMGVYDFKVSAAEFKYMVANGLHAPHGPFDALWLSFKLLGIGGDRCIKISTVQSKLTDLKCTNVVLLG
CLSSMNIAANSSEWAYCVDLHNKINLCDDPEKAQSMLLALLAFFLSKHSDFGLDGLIDSYFDNSSTEQ
SVASSFVSMPSYIAYENARQAYEDAIANGSSSQLIKQLKRAMNIAKSEFDHEISVQKKINRMAEQAAT
QMYKEARSVNRKSKVISAMHSLLFGMLRRLDMSSVETVLNLARDGVVPLSVIPATSASKLTIVSPDLE
SYSKIVCDGSVHYAGVVWTLNDVKDNDGRPVHVKEITKENVETLTWPLILNCERVVKLQNNEIMPGKL
KQKPMKAEGDGGVLGDGNALYNTEGGKTFMYAYISNKADLKFVKWEYEGGCNTIELDSPCRFMVETPN
GPQVKYLYFVKNLNTLRRGAVLGFIGATIRLQAGKQTELAVNSGLLTACAFSVDPATTYLEAVKHGAK
PVSNCIKMLSNGAGNGQAITTSVDANTNQDSYGGASICLYCRAHVPHPSMDGYCKFKGKCVQVPIGCL
DPIRFCLENNVCNVCGCWLGHGCACDRTTIQSVDISYLNEQGVLVQLDRARGSSAARLEPCNGTDIDK
CVRAFDIYNKNVSFLGKCLKMNCVRFKNADLKDGYFVIKRCTKSVMEHEQSMYNLLNFSGALAEHDFF
TWKDGRVIYGNVSRHNLTKYTMMDLVYAMRNFDEQNCDVLKEVLVLTGCCDNSYFDSKGWYDPVENED
IHRVYASLGKIVARAMLKCVALCDAMVAKGVVGVLTLDNQDLNGNFYDFGDFVVSLPNMGVPCCTSYY
SYMMPIMGLTNCLASECFVKSDIFGSDFKTFDLLKYDFTEHKENLFNKYFKHWSFDYHPNCCDCYDDM
CVIHCANFNTLFATTIPGTAFGPLCRKVFIDGVPLVTTAGYHFKQLGLVWNKDVNTHSVRLTITELLQ
FVTDPSLIIASSPALVDQRTICFSVAALSTGLTNQVVKPGHFNEEFYNFLRLRGFFDEGSELTLKHFF
FAQNGDAAVKDFDFYRYNKPTILDICQARVTYKIVSRYFDIYEGGCIKACEVVVTNLNKSAGWPLNKF
GKASLYYESISYEEQDALFALTKRNVLPTMTQLNLKYAISGKERARTVGGVSLLSTMTTRQYHQKHLK
SIVNTRNATVVIGTTKFYGGWNNMLRTLIDGVENPMLMGWDYPKCDRALPNMIRMISAMVLGSKHVNC
CTATDRFYRLGNELAQVLTEVVYSNGGFYFKPGGTTSGDASTAYANSIFNIFQAVSSNINRLLSVPSD
SCNNVNVRDLQRRLYDNCYRLTSVEESFIEDYYGYLRKHFSMMILSDDGVVCYNKDYAELGYIADISA
FKATLYYQNNVFMSTSKCWVEEDLTKGPHEFCSQHTMQIVDKDGTYYLPYPDPSRILSAGVFVDDVVK
TDAVVLLERYVSLAIDAYPLSKHPNSEYRKVFYVLLDWVKHLNKNLNEGVLESFSVTLLDNQEDKFWC
EDFYASMYENSTILQAAGLCVVCGSQTVLRCGDCLRKPMLCTKCAYDHVFGTDHKFILAITPYVCNAS
GCGVSDVKKLYLGGLNYYCTNHKPQLSFPLCSAGNIFGLYKNSATGSLDVEVFNRLATSDWTDVRDYK
LANDVKDTLRLFAAETIKAKEESVKSSYAFATLKEVVGPKELLLSWESGKVKPPLNRNSVFTCFQISK
DSKFQIGEFIFEKVEYGSDTVTYKSTVTTKLVPGMIFVLTSHNVQPLRAPTIANQEKYSSIYKLHPAF
NVSDAYANLVPYYQLIGKQKITTIQGPPGSGKSHCSIGLGLYYPGARIVFVACAHAAVDSLCAKAMTV
YSIDKCTRIIPARARVECYSGFKPNNTSAQYIFSTVNALPECNADIVVVDEVSMCTNYDLSVINQRLS
YKHIVYVGDPQQLPAPRVMITKGVMEPVDYNVVTQRMCAIGPDVFLHKCYRCPAEIVIQFLNLFMRTS
LSLLNLLVNSVLKSFLRVMYKVDNGSSINRKQLEIVKLFLVKNPSWSKAVFISPYNSQNYVASRFLGL
QIQTVDSSQGSEYDYVIYAQTSDTAHACNVNRFNVAITRAKKGIFCVMCDKTLFDSLKFFEIKHADLH
SSQVCGLFKNCTRTPLNLPPTHAHTFLSLSDQFKTTGDLAVQIGSNNVCTYEHVISFMGFRFDISIPG
SHSLFCTRDFAIRNVRGWLGMDVESAHVCGDNIGTNVPLQVGFSNGVNFVVQTEGCVSTNFGDVIKPV
CAKSPPGEQFRHLIPLLRKGQPWLIVRRRIVQMISDYLSNLSDILVFVLWAGSLELTTMRYFVKIGPI
KYCYCGNFATCYNSVSNEYCCFKHALGCDYVYNPYAFDIQQWGYVGSLSQNHHTFCNIHRNEHDASGD
AVMTRCLAVHDCFVKNVDWTVTYPFIANEKFINGCGRNVQGHVVRAALKLYKPSVIHDIGNPKGVRCA

Figure 21 (Cont.)

VTDAKWYCYDKQPVNSNVKLLDYDYATHGQLDGLCLFWNCNVDMYPEFSIVCRFDTRTRSVFNLEGVN
GGSLYVNKHAFHTPAYDKRAFVKLKPMPFFYFDDSDCDVVQEQVNYVPLRASSCVTRCNIGGAVCSKH
ANLYQKYVEAYNTFTQAGFNIWVPHSFDVYNLWQIFIETNLQSLENIAFNVVKKGCFTGVDGELPVAV
VNDKVFVRYGDVDNLVFTNKTTLPTNVAFELFAKRKMGLTPPLSILKNLGVVATYKFVLWDYEAERPF
TSYTKSVCKYTDFNEDVCVCFDNSIQGSYERFTLTTNAVLFSTVVIKNLTPIKLNFGMLNGMPVSSIK
GDKGVEKLVNWYIYVRKNGQFQDHYDGFYTQGRNLSDFTPRSDMEYDFLNMDMGVFINKYGLEDFNF**E**
HVVYGDVSKTT**LGGLHLLI**SQFRLSKMGVLKADDFVTASDTTLRCCTVTYLNELSS**K**V**VCTY**MDLLLD
DFVTILKSLDLGVISKVHEVIIDNKPYRWMLWCKDNHLSTFYPQLQSAEWKCGYAMPQIYKLQRMCLE
PCNLYNYGAGIKLPSGIML**NVV**V**KYTQLCQYL**NSTTMCVPHNMR**VLHYGAGS**D**K**GVAPGTTVLKRWLPP
DAIIIDNDINDYVSDADFSITGDCATVYLED**KFDLLISDMYD**GRIKFCDGENVSKDGFFTYLNGVIRE
K**LAIGGSVAIKITE**YSWNKYLYELIQRFA**FWTLFCT**S**VNTSSSE**A**FLIG**INYLGDFIQGPFIAGNTVH
ANYIFWRNSTIMSLSYNSVLDLSKFECKHKATVVVTLKDSDVNDMVLSLIKSGRLLLRNNGRFGGFSN
HLVSTK

Figure 21 (Cont.)

Annotated putative proteolytic cleavage products ORF 1ab

Adenosine diphosphate-ribose 1'- phosphatase

SNNPQCAIVQASESKVLLERFLPKCPEILLSIDDGHLWNLFVEKFNFVTDWLKTLKLTLTSNGLLGNC
AKRFRRVLVKLLDVYNGFLETVCSVAYTAGVCIKYYAVNVPYVVISGFVSRVIRRERCDMTFPCVSCV
TFFYEFLDTCFGVSKPNAIDVEHLELKETVFVEPKDGGQFFVSGDYLWYVVDDIYYPASCNGVLPVAF
TKLAGGKISFSDDVIVHDVEPTHKVKLIFEFEDDVVTSLCKKSFGKSIIYTGDWEGLHEVLTSAMNVI
GQHIKLPQFYIYDEEGGYDVSKPVMISQWPISNDSNGCVVEASTDFHQLECIVDDSVREEVDIIEQPF
EEVEHVLSIKQPFSFSFRDELGVRVLDQSDNNCWISTTLVQLQLTKLLDDSIEMQLFKVGKVDSIVQK
CYELSHLISGSLGDSGKLLSELLKEKYTCSITFEMSCDCGKKFDDQVGCLFWIMPYTKLFQKGECCIC
HKMQTYKLVSMKGTGVFVQDPAPIDIDAFPVKPICSSVYLGVKGSGHYQTNLYSFNKAIDGFGVFDIK
NSSVNTVCFVDVDFHSVEIEAGEVKPFAVYKNVKFYLGDISHLVNCVSFDFVVNAANENLLHGGGVAR
AIDILTEGQLQSLSKDYISSNGPLKVGAGVMLECEKFNVFNVVGPRTGKHEHSLLVEAYNSILFENGI
PLMPLLSCGIFGVRIENSLKALFSCDINKPLQVFVYSSNEEQAVLKFLDGLDLTPVIDDVDVVKPFRV
EGNFSFFDCGVNALDGDIYLLFTNSILMLDKQGQLLDTKLNGILQQAALDYLATVKTVPAGNLVKLFV
ESCTIYMCVVPSINDLSFDKNLGRCVRKLNRLKTCVIANVPAIDVLKKLLSSLTLTVKFVVESNVMDV
NDCFKNDNVVLKITEDGINVKDVVVESSKSLGKQLGVVSDGVDSFEGVLPINTDTVLSVAPEVDWVAF
YGFEKAALFASLDVKPYGYPNDFVGGFRVLGTTDNNCWVNATCIILQYLKPTFKSKGLNVLWNKFVTG
DVGPFVSFIYFITMSSKGQKGDAEEALSKLSEYLISDSIVTLEQYSTCDICKSTVVEVKSAIVCASVL
KDGCDVGFCPHRHKLRSRVKFVNGRVVITNVGEPIISQPSKLLNGIAYTTFSGSFDNGHYVVYDAANN
AVYDGARLFSSDLSTLAVTAIVVVGGCVTSNVPTIVSEKISVMDKLDTGAQKFFQFGDFVMNNIVLFL
TWLLSMFSLLRTSIMKHDIKVIAKAPKRTGVILTRSFKYNIRSALFVIKQKWCVIVTLFKFLLLLYAI
YALVFMIVQFSPFNSLLCGDIVSGYEKSTFNKDIYCGNSMVCKMCLFSYQEFNDLDHTSLVWKHIRDP
ILISLQPFVILVILLIFGNMYLRFGLLYFVAQFISTFGSFLGFHQKQWFLHFVPFDVLCNEFLATFIV
CKIVLFVRHIIVGCNNADCVACSKSARLKRVPLQTIINGMHKSFYVNANGGTCFCNKHNFFCVNCDSF
GPGNTFINGDIARELGNVVKTAVQPTAPAYVIIDKVDFVNGFYRLYSGDTFWRYDFDITESKYSCKEV
LKNCNVLENFIVYNNSGSNITQIKNACVYFSQLLCEPIKLVNSELLSTLSVDFNGVLHKAYVDVLCNS
FFKELTANMSMAECKATLGLTVSDDDFVSAVANAHRYDVLLSDLSFNNFFISYAKPEDKLSVYDIACC
MRAGSKVVNHNVLIKESIPIVWGVKDFNTLSQEGKKYLVKTTKAKGLTFLLTFNDNQAITQVPATSIV
AKQGAGFKRTYNFLWYVCLFVVALFIGVSFIDYTTTVTSFHGYDFKYIENGQLKVFEAPLHCVRNVFD
NFNQWHEAKFGVVTTNSDKCPIVVGVSERINVVPGVPTNVYLVGKTLVFTLQAAFGNTGVCYDFDGVT
TSDKCIFNSACTRLEGLGGDNVYCYNTDLIEGSKPYSTLQPNAYYKYDAKNYVRFPEILARGFGLRTI
RTLATRYCRVGECRDSHKGVCFGFDKWYVNDGRVDDGYICGDGLIDLLVNVLSIFSSSFSVVAMSGHM
LFNFLFAAFITFLCFLVTKFKRVFGDLSYGVFTVVCATLINNISYVVTQNLFFMLLYAILYFVFTRTV
RYAWIWHIAYIVAYFLLIPWWLLTWFSFAAFLELLPNVFKLKISTQLFEGDKFIGTFESAAAGTFVLD
MRSYERLINTISPEKLKNYAASYNKYKYYSGSASEADYRCACYAHLAKAMLDYAKDHNDMLYSPPTIS
YNSTLQ

3Cl^{pro} Coronavirus polyprotein processing endoprotease

SGLKKMAQPSGCVERCVVRVCYGSTVLNGVWLGDTVTCPRHVIAPSTTVLIDYDHAYSTMRLHNFSVS
HNGVFLGVVGVTMHGSVLRIKVSQSNVHTPKHVFKTLKPGDSFNILACYEGIASGVFGVNLRTNFTIK
GSFINGACGSPGYNVRNDGTVEFCYLHQIELGSGAHVGSDFTGSVYGNFDDQPSLQVESANLMLSDNV
VAFLYAALLNGCRWWLCSTRVNVDGFNEWAMANGYTSVSSVECYSILAAKTGVSVEQLLASIQHLHEG
FGGKNILGYSSLCDEFTLAEVVKQMYGVNLQ

RNA dependant RNA polymerase (pfam00680)

AGKQTELAVNSGLLTACAFSVDPATTYLEAVKHGAKPVSNCIKMLSNGAGNGQAITTSVDANTNQDSY
GGASICLYCRAHVPHPSMDGYCKFKGKCVQVPIGCLDPIRFCLENNVCNVCGCWLGHGCACDRTTIQS
VDISYLNEQGVLVQLDRARGSSAARLEPCNGTDIDKCVRAFDIYNKNVSFLGKCLKMNCVRFKNADLK
DGYFVIKRCTKSVMEHEQSMYNLLNFSGALAEHDFFTWKDGRVIYGNVSRHNLTKYTMMDLVYAMRNF
DEQNCDVLKEVLVLTGCCDNSYFDSKGWYDPVENEDIHRVYASLGKIVARAMLKCVALCDAMVAKGVV
GVLTLDNQDLNGNFYDFGDFVVSLPNMGVPCCTSYYSYMMPIMGLTNCLASECFVKSDIFGSDFKTFD
LLKYDFTEHKENLFNKYFKHWSFDYHPNCCDCYDDMCVIHCANFNTLFATTIPGTAFGPLCRKVFIDG

Figure 21 (Cont.)

VPLVTTAGYHFKQLGLVWNKDVNTHSVRLTITELLQFVTDPSLIIASSPALVDQRTICFSVAALSTGL
TNQVVKPGHFNEEFYNFLRLRGFFDEGSELTLKHFFFAQNGDAAVKDFDFYRYNKPTILDICQARVTY
KIVSRYFDIYEGGCIKACEVVVTNLNKSAGWPLNKFGKASLYYESISYEEQDALFALTKRNVLPTMTQ
LNLKYAISGKERARTVGGVSLLSTMTTRQYHQKHLKSIVNTRNATVVIGTTKFYGGWNNMLRTLIDGV
ENPMLMGWDYPKCDRALPNMIRMISAMVLGSKHVNCCTATDRFYRLGNELAQVLTEVVYSNGGFYFKP
GGTTSGDASTAYANSIFNIFQAVSSNINRLLSVPSDSCNNVNVRDLQRRLYDNCYRLTSVEESFIEDY
YGYLRKHFSMMILSDDGVVCYNKDYAELGYIADISAFKATLYYQNNVFMSTSKCWVEEDLTKGPHEFC
SQHTMQIVDKDGTYYLPYPDPSRILSAGVFVDDVVKTDAVVLLERYVSLAIDAYPLSKHPNSEYRKVF
YVLLDWVKHLNKNLNEGVLESFSVTLLDNQEDKFWCEDFYASMYENSTIEQ

ExoN 3'to 5' Exonuclease and helicase
The bold underlined amino acid residues are conserved in orthologous cellular and viral enzymes.

AAGLCVVCGSQTVLRCGDCLRKPMLCTKCAYDHVFGTDHKFILAITPYVCNASGCGVSDVKKLYLGGL
NYYCTNHKPQLSFPLCSAGNIFGLYKNSATGSLDVEVFNRLATSDWTDVRDYKLANDVKDTLRLFAAE
TIKAKEESVKSSYAFATLKEVVGPKELLLSWESGKVKPPLNRNSVFTCFQISKDSKFQIGEFIFEKVE
YGSDTVTYKSTVTTKLVPGMIFVLTSHNVQPLRAPTIANQEKYSSIYKLHPAFNVSDAYANLVPYYQL
IGKQKITTIQGPPGSGKSHCSIGLGLYYPGARIVFVACAHAAVDSLCAKAMTVYSIDKCTRIIPARAR
VECYSGFKPNNTSAQYIFSTVNALPECNADIVVVDEVSMCTNYDLSVINQRLSYKHIVYVGDPQQLPA
PRVMITKGVMEPVDYNVVTQRMCAIGPDVFLHKCYRCPAEIVIQFLNLFMRTSLSLLNLLVNSVLKSF
LRVMYKVDNGSSINRKQLEIVKLFLVKNPSWSKAVF**ISPYNS**QNYVASRFLGLQIQTVDSSQGSEYDY
VIYAQTSDTAHACNVNRFNVAITRAKKGIFCVMCDKTLFDSLKFFEIKHADLHSSQVCGLFKNCTRTP
LNLPPTHAHTFLSLSDQFKTTGDLAVQIGSNNVCTYEHVISFMGFRFDISIPGSHSLFCTRDFAIRNV
RG**WLGMDVESAH**VCGDNIGTNVPLQVGFSNGVNFVVQTEGCVSTNFGDVIKPVCAKSPPGEQFRHLIP
LLRKGQPWLIVRRRIVQMISDYLSNLSDILVFVLWAGSLELTTMRYFVKI**G**PIKY**C**Y**CG**NF**ATCYNS**V
SNE**Y**C**C**F**K**H**A**L**G**C**DYVYNP**YA**FDIQ**QWGYVGSLSQNHHTFCNIHRNE**HDASG**D**AVMTRCLA**VHDCFVK
NVDWTVTYPFIANEKFINGCGRNVQGHVVRAALKLYKPSVIHDIGNPKGVRCAVTDAKWYCYDKQPVN
SNVKLLDYDYATHGQLDGLCLFWNCNVDMYPEFSIVCRFDTRTRSVFNLEGVNGGSLYVNKHAFHTPA
YDKRAFVKLKPMPFFYFDDSDCDVVQEQVNYVPLRASSCVTRCNIGGAVCSKHANLYQKYVEAYNTFT
QAGFNIWVPHSFDVYNLWQIFIETN**LQ**

XendoU (homolog of) polyU-specific endoribonuclease
SLENIAFNVVKKGCFTGVDGELPVAVVNDKVFVRYGDVDNLVFTNKTTLPTNVAFELFAKRKMGLTPP
LSILKNLGVVATYKFVLWDYEAERPFTSYTKSVCKYTDFNEDVCVCFDNSIQGSYERFTLTTNAVLFS
TVVIKNLTPIKLNFGMLNGMPVSSIKGDKGVEKLVNWYIYVRKNGQFQDHYDGFYTQGRNLSDFTPRS
DMEYDFLNMDMGVFINKYGLEDFNF**EHVVYGD**VSKTT**LGGLHLLI**SQFRLSKMGVLKADDFVTASDTT
LRCCTVTYLNELSS**K**V**VCTY**MDLLLDDFVTILKSLDLGVISKVHEVIIDNKPYRWMLWCKDNHLSTFY
PQ**LQ**

2'-O-MT 2: S-adenosylmethionine-dependant ribose 2'-orthomethyltransferase
Plays a role in the methylation of cap structure (GpppNm) at the 5'end of the viral RNA. Antiviral compounds inhibiting this transfer of methyl groups to reaction (carboxylic adenosine analogs *e.g.* Neoplanocin A and 3-deazaneoplancin A) interfere with expression of viral proteins. Again the underlined residues in bold print are conserved

SAEWKCGYAMPQIYKLQRMCLEPCNLYNYGAGIKLPSGIML**NVV**K**YTQLCQYL**NSTTMCVPHNMR**VLH**
YGAGSD**K**GVAPGTTVLKRWLPPDAIIIDNDINDYVSDADFSITGDCATVYLED**KFDLLISDMYD**GRIK
FCDGENVSKDGFFTYLNGVIREK**LAIGGSVAIKITE**YSWNKYLYELIQRFA**FWTLFCTSVNTSSSEAF**
LIGINYLGDFIQGPFIAGNTVHANYIFWRNSTIMSLSYNSVLDLSKFECKHKATVVVTLKDSDVNDMV
LSLIKSGRLLLRNNGRFGGFSNHLVSTK

Figure 22

ORF-2 Spike protein/S-gene

MKLFLILLVLPLASC

FFTCNSNANLSMLQLGVPDNSSTIVTGLLPTHWFCANQSTSVYSA
NGFFYIDVGNHRSAFALHTGYYDANQYYIYVTNEIGLNASVTLKICKFSRNTTFDFLSNA
SSSFDCIVNLLFTEQLGAPLGITISGETVRLHLYNVTRTFYVPAAYKLTKLSVKCYFNYS
CVFSVVNATVTVNVTTHNGRVVNYTVCDDCNGYTDNIFSVQQDGRIPNGFPFNNWFLLTN
GSTLVDGVSRLYQPLRLTCLWPVPGLKSSTGFVYFNATGSDVNCNGYQHNSVVDVMRYNL
NFSANSLDNLKSGVIVFKTLQYDVLFYCSNSSSGVLDTTIPFGPSSQPYYCFINSTINTT
HVSTFVGILPPTVREIVVARTGQFYINGFKYFDLGFIEAVNFNVTTASATDFWTVAFATF
VDVLVNVSATNIQNLLYCDSPFEKLQCEHLQFGLQDGFYSANFLDDNVLPETYVALPIYY
QHTDINFTATASFGGSCYVCKPHQVNISLNGNTSVCVRTSHFSIRYIYNRVKSGSPGDSS
WHIYLKSGTCPFSFSKLNNFQKFKTICFSTVEVPGSCNFPLEATWHYTSYTIVGALYVTW
SEGNSITGVPYPVSGIREFSNLVLNNCTKYNIYDYVGTGIIRSSNQSLAGGITYVSNSGN
LLGFKNVSTGNIFIVTPCNQPDQVAVYQQSIIGAMTAVNESRYGLQNLLQLPNFYYVSNG
GNNCTTAVMTYSNFGICADGSLIPVRPRNSSDNGISAIITANLSIPSNWTTSVQVEYLQI
TSTPIVVDCATYVCNGNPRCKNLLKQYTSACKTIEDALRLSAHLETNDVSSMLTFDSNAF
SLANVTSFGDYNLSSVLPQRNIRSSRIAGRSALEDLLFSKVVTSGLGTVDVDYKSCTKGL
SIADLACAQYYNGIMVLPGVADAERMAMYTGSLIGGMVLGGLTSAAAIPFSLALQARLNY
VALQTDVLQENQKILAASFNKAINNIVASFSSVNDAITQTAEAIHTVTIALNKIQDVVNQ
QGSALNHLTSQLRHNFQAISNSIQAIYDRLDSIQADQQVDRLITGRLAALNAFVSQVLNK
YTEVRGSRRLAQQKINECVKSQSNRYGFCGNGTHIFSIVNSAPDGLLFLHTVLLPTDYKN
VKAWSGICVDGIYGYVLRQPNLVLYSDNGVFRVTSRVMFQPRLPVLSDFVQIYNCNVTFV
NISRVELHTVIPDYVDVNKTLQEFAQNLPKYVKPNFDLTPFNLTYLNLSSELKQLEAKTA
SLFQTTVELQGLIDQINSTYVDLKLLNRFENYIKWPWWVWLIISVVFVVLLSLLVFCCLS
TGCCGCCNCLTSSMRGCCDCGSTKLPYYEFEKVHVQ

Figure 23

ORF-4 Corona virus envelope protein/E-gene

MFLRLIDDNGIVLNSILWLLVMIFFFVLAMTFIKLIQLCFTCHYFFSRTLYQPVYKIFLA
YQDYMQIAPVPAEVLNV

ORF-5 pfam01635, Corona_M, Coronavirus M matrix/glycoprotein.

MSNSSVPLLEVYVHLRNWNFSWNLILTLFIVVLQYGHYKYSRLLYGLKMSVLWCLWPLVLA

LSIFDCFVNFNVDWVFFGFSILMSIITLCLWVMYFVNSFRLWRRVKTFWAFNPETNAII
SLQVYGHNYYLPVMAAPTGVTLTLLSGVLLVDGHKIATRVQVGQLPKYVIVATPSTTIVC
DRVGRSVNETSQTGWAFYVRAKHGDFSGVASQEGVLSEREKLLHLI

ORF-6 Pfam 00937, Coronavirus nucleocapsid protein

MASVNWADDRAARKKFPPPSFYMPLLVSSDKAPYRVIPRNLVPIGKGNKDEQIGYWNVQE
RWRMRRGQRVDLPPKVHFYYLGTGPHKDLKFRQRSDGVVWVAKEGAKTVNTSLGNRKRNQ
KPLEPKFSIALPPELSVVEFEDRSNNSSRASSRSSTRNNSRDSSRSTSRQQSRTRSDSNQ
SSSDLVAAVTLALKNLGFDNQSKSPSSSGTSTPKKPNKPLSQPRADKPSQLKKPRWKRVP
TREENVIQCFGPRDFNHNMGDSDLVQNGVDAKGFPQLAELIPNQAALFFDSEVSTDEVGD
NVQITYTYKMLVAKDNKNLPKFIEQISAFTKPSSIKEMQSQSSHVAQNTVLNASIPESKP
LADDDSAIIEIVNEVLH

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which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 04 07 5050

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	DATABASE EMBL [Online] EBI; 11 July 2001 (2001-07-11), THIEL ET AL.: "Human coronavirus 229E" XP002290973 Database accession no. AF304460 Overall about 70% homologous to SEQ ID NO:55 * abstract * -----	1,2,4,9, 14,15, 18,19, 21,27, 35,39, 40,53-55	C12N7/00 C07K14/165 A61K39/215 C12Q1/68 G01N33/569 C07K16/10
X	DATABASE SWISSPROT [Online] EBI; 15 September 2003 (2003-09-15), THIEL V ET AL.: "Human conoravirus 229E replicase polyprotein 1ab" XP002290974 Database accession no. Q05002 54%-83% homologous to SEQ ID NOs:56-63 * abstract * -----	5,6,8, 21,24, 25,29, 33,36,37	
	-/--		

TECHNICAL FIELDS SEARCHED (Int.Cl.7)

C12N
C07K
A61K

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

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Place of search	Date of completion of the search	Examiner
The Hague	4 August 2004	Brouns, G

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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Application Number
EP 04 07 5050

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	DATABASE SWISS-PROT [Online] EBI; 15 August 1999 (1999-08-15), RAABE T ET AL.: "E2 glycoprotein precursor (spike glycoprotein) (peplomer protein)" XP002275246 Database accession no. P15423 65% homologous to 1168 amino acids of SEQ ID NO:64 * abstract * ----- DATABASE SWISS-PROT [Online] EBI; 15 September 2003 (2003-09-15), RAABE T AND SIDDELL S: "Human coronavirus 229E envelope protein" XP002290975 Database accession no. P19741 47% identical to SEQ ID NO:65 * abstract * ----- DATABASE SWISS-PROT [Online] EBI; 15 September 2003 (2003-09-15), SCHREIBER ET AL.: "Human coronavirus 229E nucleocapsid protein" XP002290976 Database accession no. P15130 44% identical to SEQ ID NO:67 * abstract * ----- -/--	5,6,8, 21,24, 25,29, 33,36,37 5,6,8, 21,24, 25,29, 33,36,37 5,6,8, 21,24, 25,29, 33,36,37	TECHNICAL FIELDS SEARCHED (Int.Cl.7)

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EPO FORM 1503 03.82 (P04C10)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number EP 04 07 5050

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
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X	ANAND KANCHAN ET AL: "Coronavirus main proteinase (3CLpro) structure: Basis for design of anti-SARS drugs." SCIENCE (WASHINGTON D C), vol. 300, no. 5626, 13 June 2003 (2003-06-13), pages 1763-1767, XP002291115 ISSN: 0036-8075 * page 1765, right-hand column * * page 1766, right-hand column, paragraph 1 *	50-52	
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T	VAN DER HOEK LIA ET AL: "Identification of a new human coronavirus" NATURE MEDICINE, vol. 10, no. 4, April 2004 (2004-04), pages 368-373, XP002290972 ISSN: 1078-8956 * the whole document *	1-59	

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PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 04 07 5050

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
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T	FOUCHIER RON A M ET AL: "A previously undescribed coronavirus associated with respiratory disease in humans" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 101, no. 16, 20 April 2004 (2004-04-20), pages 6212-6216, XP002291117 ISSN: 0027-8424 * the whole document * -----	1-59	TECHNICAL FIELDS SEARCHED (Int.Cl.7)

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European Patent Office

INCOMPLETE SEARCH SHEET C

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Although claim 41 is directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.
Although claim 42 is directed to a diagnostic method practised on the human/animal body comprising a step 'obtaining a sample' (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.

Claim(s) not searched:
12,49

Reason for the limitation of the search:

The claims refer to amino acid and nucleotide sequences as depicted in figures 16-19 and/or tables 3, 7 and 10, which are considered to relate to SEQ ID NOs: 9-13, 31-35, 47-65 and 67, and on which the search has been based.

Claim 12 refers to a virus with a desirable characteristic, namely that it is 'capable of inducing' a HCoV-NL63 related disease. However, due to lack of technical features, no meaningful search for said virus is possible.
In addition, claims 17-20, 30, 31,34, 35 and 40 refer to an isolated molecule 'binding' to the virus of the invention, or a part thereof. No specific examples for said molecules are provided and the search has been limited to antibodies, antisense nucleic acid molecules and ribozymes binding to the virus, viral proteins or viral nucleic acids of the invention. Since the essential technical and structural features of said molecules are well known to the skilled person, said molecules are considered to be sufficiently disclosed and supported. In addition, the 'compound' of claim 49 has not been defined by searchable technical features and claims 50-52 have only been searched in as far as said compound comprises an amino acid sequence YNSTLQ or the hexapeptides of claim 52 defined by SEQ ID NOs:1 and 2.

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 04 07 5050

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

04-08-2004

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EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82